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PhD Thesis

STRUCTURAL OPTIMIZATION OF MONO AND MULTIVALENT GLYCOMIMETIC MANNOSE BASED DC-SIGN LIGANDS

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Summary

List of abbreviations

Ac	acetile
Ar	aromatic
ax	axial
Bn	benzyl
Boc	*tert*-butyloxycarbonyl
BSA	Bovine serum albumin
CRD	carbohydrate recognition domain
DC	dendritic cell
DCM	dichloromethane
DC-SIGN	Dendritic Cell-Specific ICAM-3 Grabbing Nonintegrin
DHB	2,5-dihydroxybenzoic acid
DIPEA	diisopropylethylamine
DMA	N,N'-dimethylacetamide
DMF	N,N'-dimethylformamide
DMSO	dimethylsulfoxide
EA	ethyl acetate
ECD	extracellular domain
EDC	N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride
ELISA	Enzyme-Linked ImmunoSorbent Assay
eq	equatorial
ESI-MS	electronspray ionization mass spectroscopy
HCCA	α-cyano-4-hydroxycinnamic acid
hex	hexane
HRMS	high resolution mass spectroscopy
IC50	median inhibition concentration
ICAM-3	Intercellular Adhesion Molecule 3
J	coupling constant

LC-Mass	liquid chromatography-mass spectroscopy
MALDI	matrix-assisted laser desorption/ionization spectrometry
Man	D-mannose
MCPBA	m-chloroperbenzoic acid
Me	methyl
NMR	nuclear magnetic resonance
PAMAM	poly(amido amine)
PAMPs	pathogen-Associated Molecular Patterns
PG	protecting group
Ph	phenyl
PRRs	pattern Recognition Receptor
quant	quantitative
rt	room temperature
SA	sinapinic acid
SPR	surface Plasmon resonance
STD	saturation transfer difference
TBAF	tetrabutylammonium fluoride
TBTA	tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine
tBu	*tert*-butyl
TFA	trifluoroacetic acid
TEA	triethylamine
THF	tetrahydrofurane
TLC	thin layer chromatography
TLRs	toll-like receptors
TMSOTf	trimethylsilyl trifluoromethanesulfonate

Chapter 1

Introduction

1.1 The role of Cell-Mediated immunity in the immune system

The immune system can be divided in two main branches, the Humoral and the Cell-mediated immunity. Humoral immunity is responsible for the production of antibodies while the cell-mediated immunity protects the body using several mechanisms. It can activate antigen-specific cytotoxic T-lymphocytes that are able to induce apoptosis in body cells displaying epitopes of foreign antigens on their surface, such as virus-infected cells, cells with intracellular bacteria, and cancer cells displaying tumour antigens. The cell-mediated immunity also activates macrophages and natural killer cells, enabling them to destroy intracellular pathogens, and it can stimulate cells to secrete a variety of cytokines that influence the function of other cells involved in adaptive and innate immune responses. Intracellular microorganisms may elicit the production of antibodies or activate specific T-cells. Activation of T-cells takes place exclusively under the so-called Major Histocompatibility Complex (MHC) restriction (Figure 1.1). The MHC is basically a set of molecules displayed on cell surfaces that are responsible for lymphocyte recognition and "antigen presentation". T-cells recognize, by the T-cell Receptor (TCR, on the T-cell surface), only specific antigenic peptides bound to an MHC molecule presented by Antigen Presenting Cells (APCs). This recognition is "MHC-restricted" because the TCR also requires interactions with MHC. Into the APCs family, among others, belong also Dendritic Cells (DCs). The MHC molecules control the immune response through recognition of "self" and "non-self" and, consequently, serve as targets in transplantation rejection. There are several classes of MHC molecules. Class I and Class II belong to a group of molecules known as the Immunoglobulin Supergene Family, which includes immunoglobulins, T-cell receptors, CD4, CD8, and others.

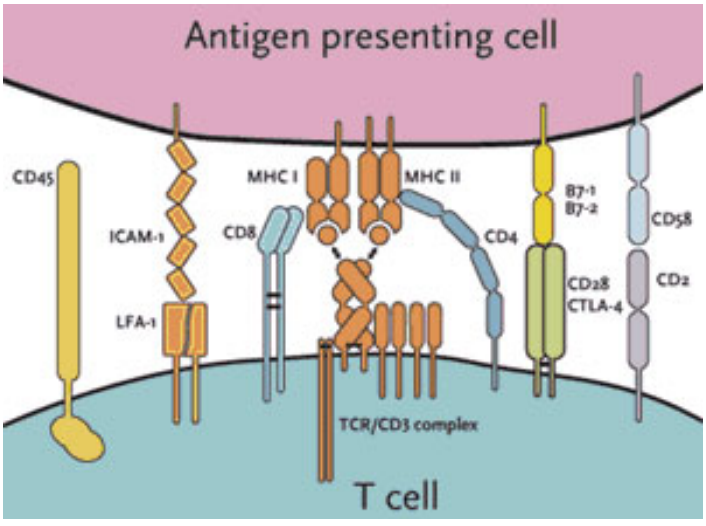


Figure 1.1 Schematic representation of the major histocompability complex (MHC)[1]

Class I molecules are found on virtually every cell in the human body and present "endogenous" antigens to cytotoxic T-cells (CTLs). An endogenous antigen might be a fragment of viral proteins or tumour proteins: presentation of such antigens would indicate internal cellular alterations that if not contained could spread throughout the body. Hence, destruction of these cells by CTLs is advantageous to the body as a whole.

Class II molecules are only found on professional APCs like B-cells, macrophages and dendritc cells and present "exogenous" antigens to helper T-cells (T_H-cells). Exogenous antigens might be fragments of bacterial cells or viruses that are engulfed and processed by e.g. a macrophage and then presented to helper T-cells. The T_H-cells, in turn, could activate B-cells to produce antibody that would lead to the destruction of the pathogen.

Professional APCs can internalize antigens very efficiently, either by phagocytosis or by receptor-mediated endocytosis. After internalisation APCs usually migrate to the lymph vessels and are carried via lymph flow to the draining lymph nodes. During the migration, DCs and other APCs undergo maturation, mainly by loosing most of their ability to further engulf pathogens, and developing an increased ability to communicate with T cells. In the lymph nodes APCs such as dendritc cells can interact with T cells.

Within the DC lysosomal compartment the internalized pathogen can be digested by proteolytic enzymes, reactive oxygen intermediates (ROI) and nitrogen monoxide (NO) into smaller pieces, and only a few of them are epitopes stable enough to migrate toward the cell surface and to be presented to T cells as MHC II complex.[2]

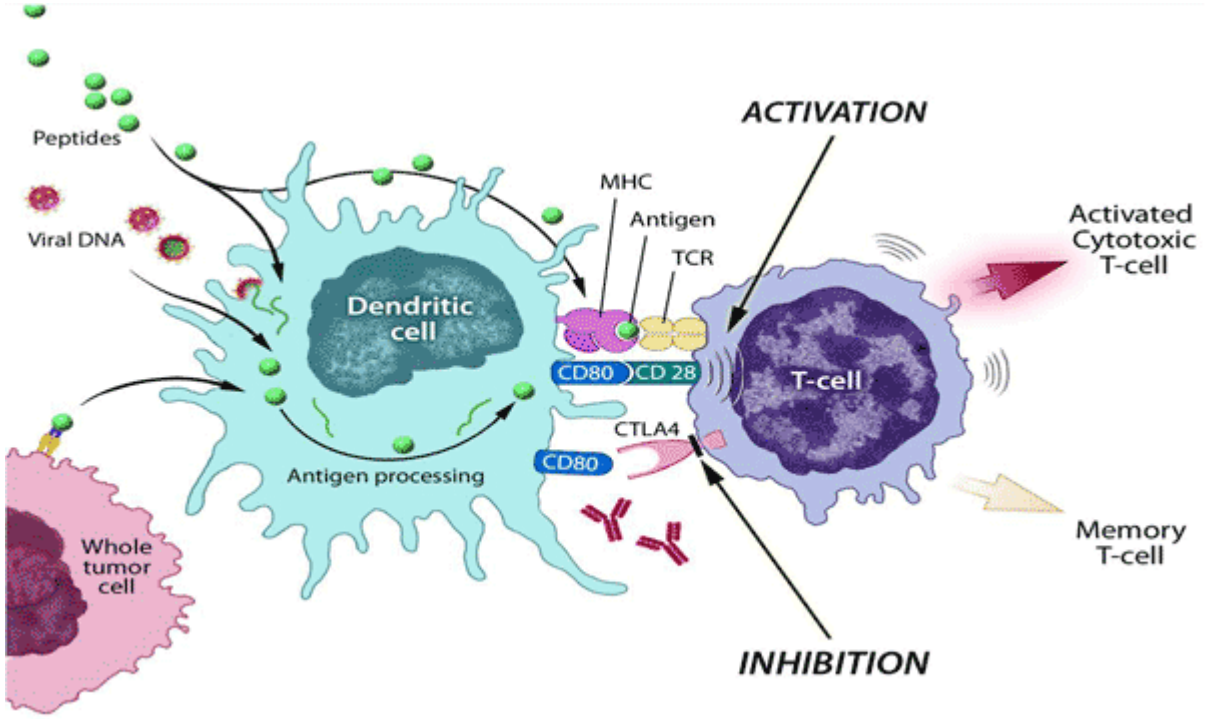


Figure 1.2 Antigen presentation of dendritic cell to the T-cell by MHC molecule[3]

DCs express a range of Pathogen-Recognition Receptors (PRRs), including Toll-like receptors (TLRs) and C-type lectins that can recognize molecular patterns expressed by pathogens.[4] PRRs recognize characteristic molecular patterns in microbial cell-wall components, such as carbohydrate structures (C-type lectins), nucleic acids (TLRs) and lipids.

The DC response is modulated depending on the type or form of a microorganism that is recognized by different TLRs and C-type lectins. TLRs relay the information about the interacting pathogen to DCs through intracellular-signalling cascades, thereby eliciting appropriate cellular processes that lead to DC maturation and the induction of inflammatory cytokines, whilst C-type lectins internalize pathogens for degradation in lysosomal compartments to enhance antigen processing and presentation by DCs. Carbohydrate structures on self glycoproteins are also recognised by C-type lectins, thus allowing tolerance to self antigens and helping to mediate cellular processes, such as cell signalling, cell adhesion and migration.

There have been described many different C-type lectins expressed by DCs, such as the mannose receptor (CD206), DEC205 (CD205), DC-SIGN (CD209), blood DC antigen 2 (BDCA2), dectin-1, DC immunoreceptor (DCIR), DC-associated lectin 1 (DCAL1), C-type lectin receptor 1 (CLEC1), Langherhans-cell-specific C-type lectin (Langherin, CD207) and DC-asialoglycoprotein receptor (DC-ASGPR) / macrophage galactose N-acetyl-galactosamine specific lectin 1 (MGL1). Many of these C-type lectins have been shown to function as antigen

receptors. Monocyte-derived DCs and interstitial DCs express the highest diversity of C-type lectins. By contrast, only a few C-type lectins have been identified on DCs from the blood and Langherhans cells. Langherhans cells specifically express Langherin, whereas plasmacytoid DCs express BDCA2 and dectin-1.

1.2 DC-SIGN (Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin)

DC-SIGN is a C-type lectin receptor (CLR) expressed exclusively on dendritic cells.[5] It fulfils several functions: as adhesive molecule it enables DC migration, pathogen/antigen recognition and antigen presentation to T-Cells. After ligand binding, DC-SIGN initiate a signal pathway, which modulates DC maturation and cytokine-expression profile.[6]

CLRs contain one or more carbohydrate recognition domains (CRDs). The CRD of DC-SIGN is a globular structure consisting of 12 β-strands, two α-helices and three disulphide bridges.[7,8] DC-SIGN also contains a neck region composed of four associated chains, each composed of seven complete and one incomplete tandem repeats, and a transmembrane region followed by a cytoplasmic tail containing recycling internalisation and intracellular signalling motifs, *i.e.* a di leucine (LL) motif, tri-acidic (EEE) clusters, and an incomplete immunoreceptor, tyrosine-based, activation motif (Figure 1.3).

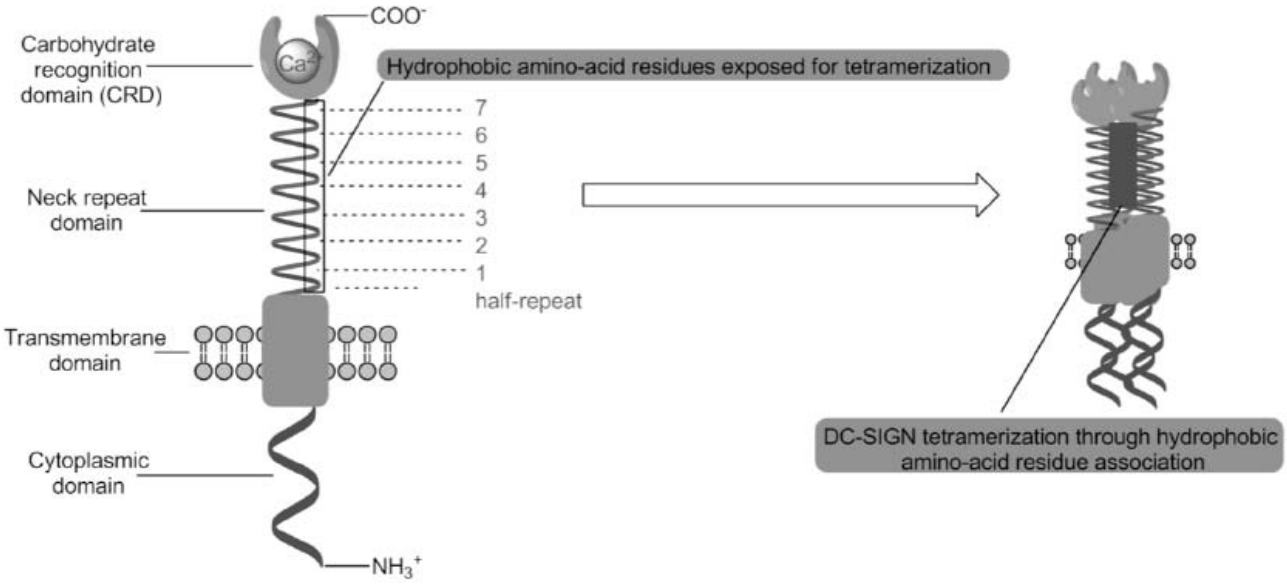


Figure 1.3 The structure of DC-SING and the tetramerisation through association of the neck domains[9]

DC-SIGN undergoes tetramerisation which is initiated by the neck domain[10] which has an impact on the binding affinity of the receptor and also provides specificity, thereby defining the set of pathogens that are recognized by DC-SIGN. The tetramerisation depends highly on pH; in acidic media the tetramer falls apart to monomers and thus pathogen release can occur in the acidic endosomal environment, where its degradation takes place.[11]

DC-SIGN recognises mannose and fucose-containing glycans which makes this receptor a target for a great number of important pathogens such as bacteria, parasites, fungi and viruses.[2,7, 12] One of the characteristics of some bacterial pathogens is that they use DC-SIGN to increase their infectivity and host survival, which leads to chronic infectious states: during the pathogen - DC-SIGN interaction an inappropriately polarized T-cell response is developed, which can not ensure complete clearance of the pathogen. An example of pathogen which uses DC to spread itself is the HIV-1 virus.[13] HIV infects DCs (in mucosal tissues and blood) which then carry the virus to the lymphoid tissue where it infects the CD4+ T cells. The first contact between DC-SIGN and HIV-1 occurs via its envelop gp120 protein, and the DCs are immature during this state. The formed DC-SIGN-HIV-1 complex is internalised to the endosomes where the acidic media causes dissociation.[14] Most of the ligands are lysed and processed via degradation pathways, but HIV-1 probably remains bound to DC-SIGN and the small amount of HIV-1 that enters DCs remain protected from the host immune system and retains its infectiveness.[15,16] HIV-1 stays hidden in multivesicular bodies for days until it reaches the T cell and infects them. However, HIV-1 adhesion to DCs may also occur in a receptor-independent way so HIV-1 may adhere to DCs by a variety of modes depending on the DC type and maturation status. A number of other pathogens besides HIV-1 bind to DCSIGN. Viruses (HCV, CMV, Dengue, Ebola, SARS-CoV, HSV, coronaviruses, H5N1, West Nile virus, measles virus), bacteria (M. Tuberculosis, H.pylori, L. interrogans), fungi (C. albicans, A.fumigatus) and several parasites (Leishmania, S. mansoni) use DC-SIGN as their main cellular entry mechanism.[4]

1.3 Langherin

As it was described in the previous section HIV-1 virus is transmitted to T-cells by DCs through DC-SIGN. However, pathogens like HIV-1 interact also with epithelial Langherhans cells (LCs), which are the first DC subset to encounter HIV-1 virus.[17] LCs interacts with the pathogen via Langherin receptor. While interaction of HIV-1 with DC-SIGN enables HIV-1 to survive a host immune system, Langherin mediates HIV-1 internalisation into Birbeck granules where viral particles are degraded,[18] thus interaction of the pathogen with Langherin is desired since it helps to prevent the infection.

Langherin is structurally similar to DC-SIGN; the extracellular domain consists of a C-type carbohydrate-recognition domain (CRD) and a neck region, which induces oligomerisation. While DC-SIGN exists as tetramer, Langherin is a trimer (Figure 1.4).[19]

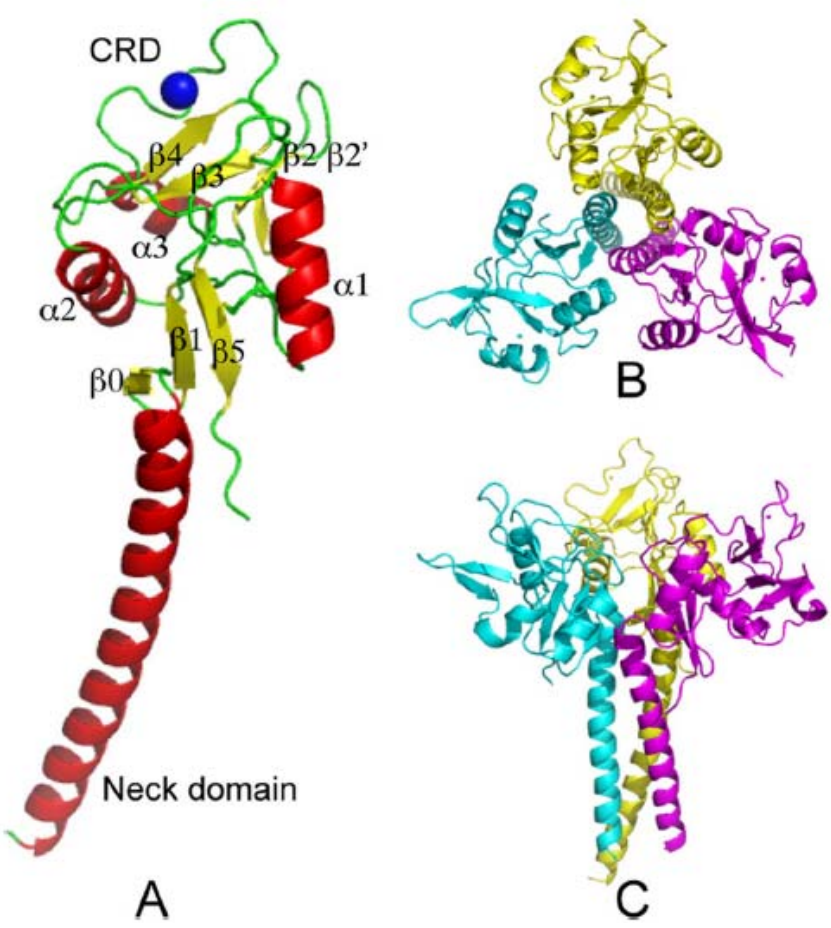


Figure 1.4 The monomeric and trimetric structure of Langherin[18]

The binding site of the CRD contains a calcium atom which can coordinate mannose or glycans containing mannoses However, as it will be discussed in chapter 2, the binding site is structurally different from the binding site of DC-SIGN,[20] allowing development of ligands which selectively binds to DC-SIGN but not Langherin.

1.4 Natural DC-SIGN ligands

Pathogens are using heavily glycosylated envelopes to bind DC-SIGN. A study from 2004 using glycan arrays probed with fluorescent-labelled DC-DIGN and DC-SIGNR showed that the most

potent fucose based natural ligands are Lewis a, b, x and y, and blood group A and B (Figure 1.5).[21]

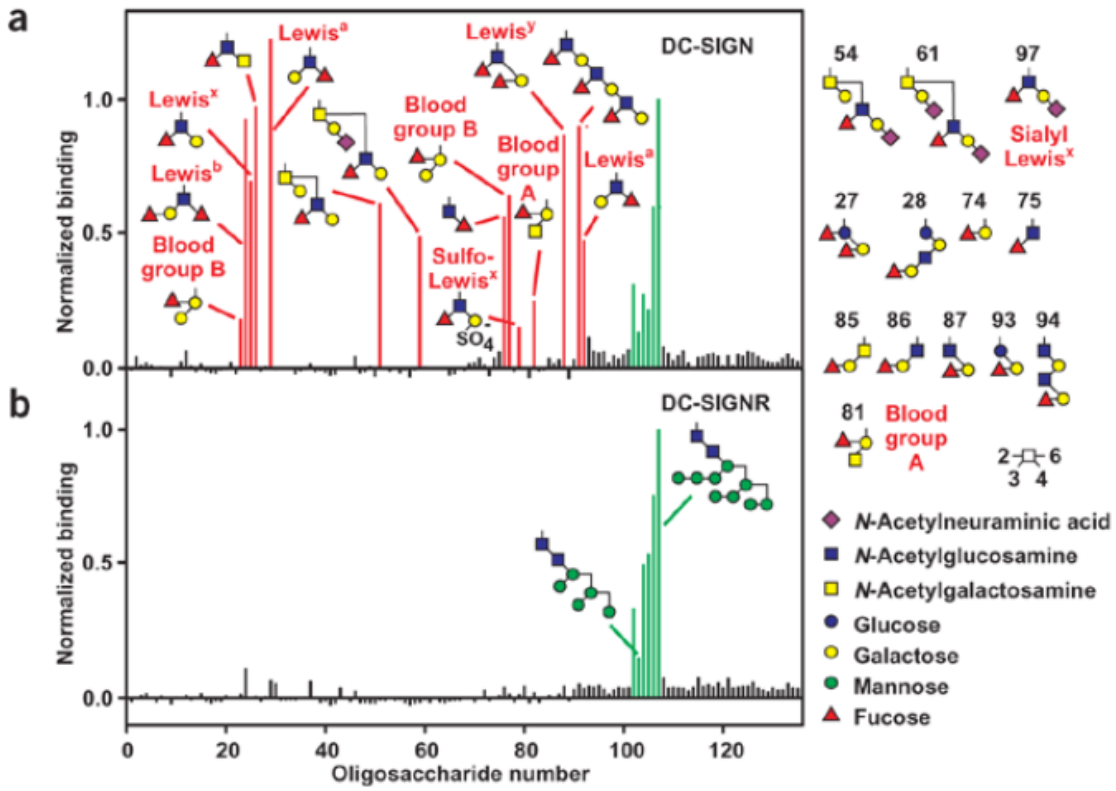
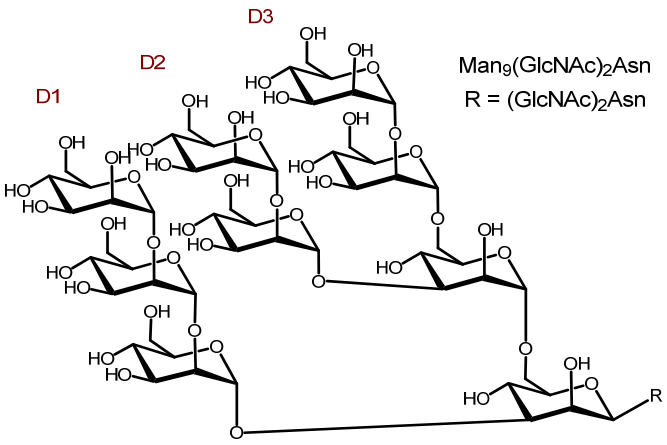


Figure 1.5 Fucose and mannose based natural oligosaccharides and their affinity to binds DC-SIGN[21]

Among the mannose based ligands, the oligomannoside Man_9 was found as progenitor of the high-Man family and exhibited the highest affinity with DC-SIGN (Scheme 1.1).



Scheme 1.1 The structure of Man_9

Another study, using a recent version of the Consortium for Functional Glycomics glycan array with human DC-SIGN, found that the three most active ligands belong to the high-Man family followed by some fucosylated Lewis-type structures (Figure 1.7).[22]

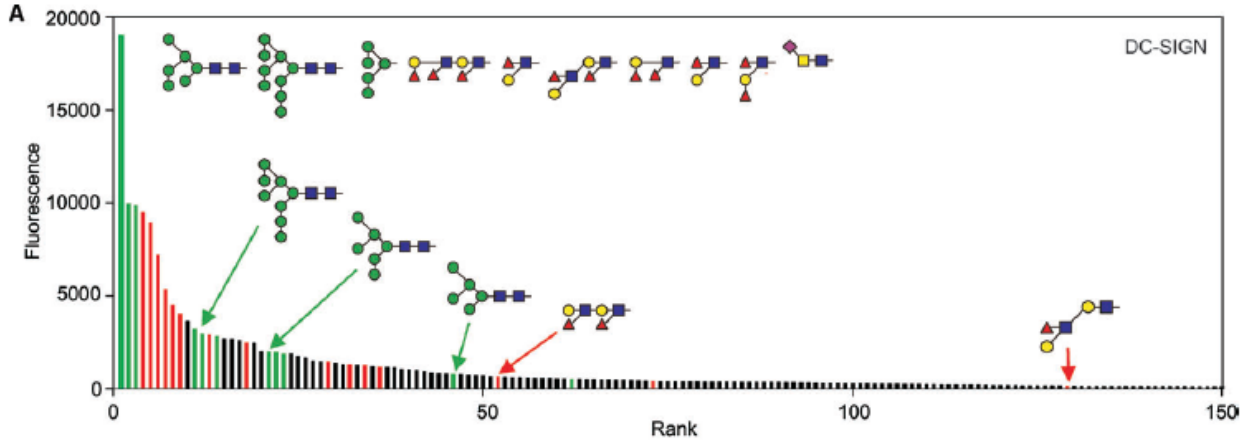
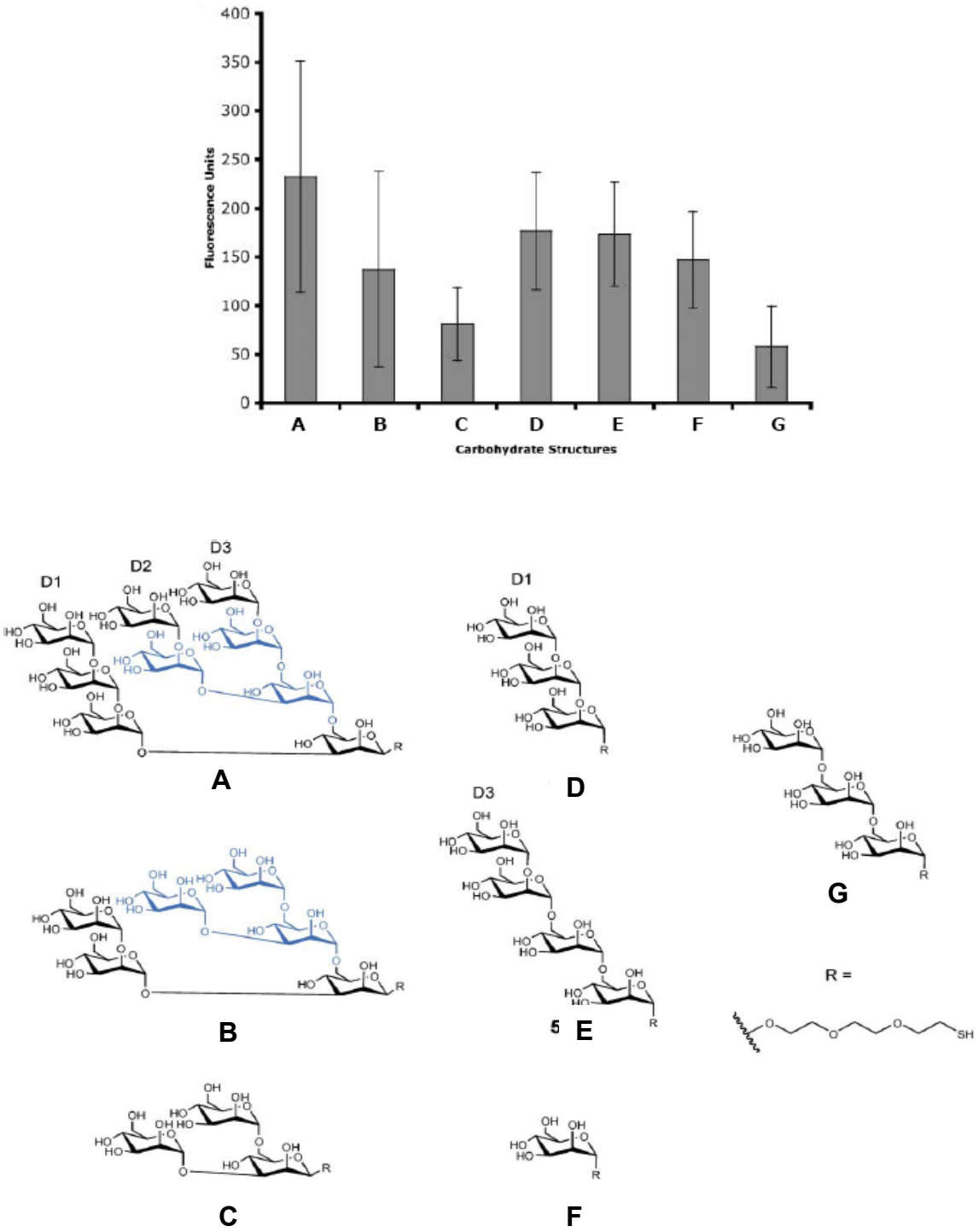


Figure 1.6 Natural DC-SIGN ligands arranged by the potency to bind DC-SIGN[22]

Binding assays to high density glycan-arrays functionalized with the complete epitope and its fragments allowed to detect that simpler linear fragments of Man_9 have similar binding affinities DC-SIGN as Man_9 itself (Scheme 1.2).[23]



Scheme 1.2 The structure of Man_9 and its fragments and their potency to bind DC-SIGN[23]

1.5 DC-SIGN as therapeutic target

As it was mentioned above, DC-SIGN binds a large number of different pathogens and some of them use this receptor as a Trojan horse to reach T-Cells and spread the infection. This suggests that DC-SIGN can be an interesting therapeutic target,[24] since inhibition of the DC-SIGN – pathogen interaction could prevent the localised infection of DCs and also the pathogen

dissemination. Numerous publications deal with DC-SIGN as a possible target for anti-infective therapy[25,26,27,28] and several distinct strategies are used to accomplish this goal:

• inhibition of pathogen binding to DC-SIGN by DC-SIGN specific ligands – small molecule DC-SIGN antagonists or mAbs against DC-SIGN.[22,29,30,31,32]

• inhibition of pathogen binding to DC-SIGN by carbohydrate-specific ligands[23]

• inhibition of pathogen binding to DC-SIGN by PAMP (Pathogen-Associated Molecular Patterns)-specific ligands/ antibodies[25]

• use of specific DC-SIGN targeted vectors that encode pathogen proteins to induce immunisation[33]

The use of small molecules as DC-SIGN antagonist can be a promising methodology, and there have been already reported many molecules with different structural properties as potential DC-SIGN ligands. The natural monovalent DC-SIGN ligands as mannose and fucose bind only with low affinity and this prevent them in use for therapeutic treatment. Moreover, the high ligand promiscuity of DC-SIGN requires specific molecules which bind selectively this target receptor. The high polarity of mono and polysaccharides is not in accordance with the common drug-like structures and therefore the design of therapeutically useful DC-SIGN antagonists is still a challenging task.

In the design of potent and selective DC-SIGN antagonists three main concepts have been used

1. the design of monovalent glycomimetics based on the DCSIGN-binding oligosaccharides[34,35,36,37,38,39,40]
2. multimeric presentation of monosaccharides/oligosaccharides or glycomimetics[41,42,43,44,40,45]
3. screening of compound libraries to obtain non-carbohydrate DC-SIGN antagonists[31,32]

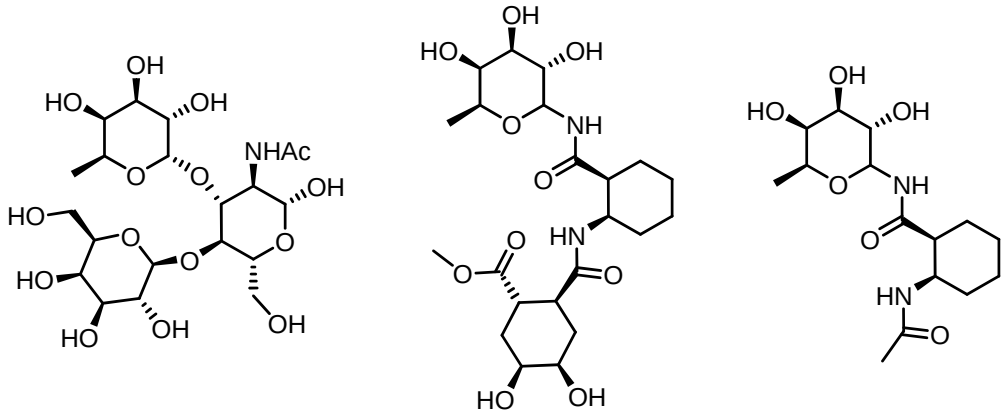
1.6 Monovalent glycomimetics as DC-SIGN ligands

The CRD of DC-SIGN contains a calcium atom which is able to coordinate mannose and fucose molecules. With proper modification of natural sugar containing ligands the affinity and selectivity towards DC-SIGN can be improved. Furthermore, by changing from sugar to “sugar like” structures higher metabolical stability against sugar hydrolysing enzymes can be achieved. In general, the monovalent sugar anchor is used unchanged (since it primarily binds to calcium) and is decorated with structures which help to gain further interaction with the binding site and/or improve its overall properties.

1.6.1 Carbohydrate based DC-SIGN ligands

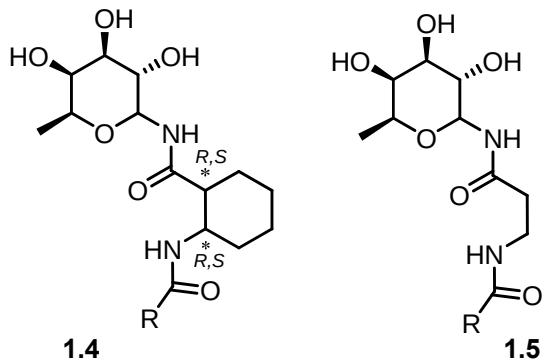
1.6.1.1 Fucose based

Bernardi *et. al*, by mimicking the Lewis-X trisacharide **1.1**, designed α-fucosylamides as DC-SIGN ligands.[35,34] The α-amidic bond is used as a surrogate for the metabolically unstable α-glycosidic bond. In the first generation of fucosyl amides the full Lewis-X mimic **1.2** showed higher affinity than the natural Lewis X. The structurally simpler mimic **1.3** was found to be almost as potent as compound **1.2** (Scheme 1.3). STD-NMR experiments showed that only the fucose part of the molecule has strong interaction with the CRD of DC-SIGN.



Scheme 1.3 Structure of natural and synthetic fucose based DC-SIGN ligands[35]

Further, a set of 30 compounds was synthesised with general structure **1.4** and binding affinities similar to compound **1.3**, regardless on the R group and configuration of the central scaffold. A replacement of the aminocyclohexanecarboxylic acid ring by the simpler and more flexible β-alanin gave compounds with general structure **1.5** which exhibited affinities similar to Lewis X (Scheme 1.4).

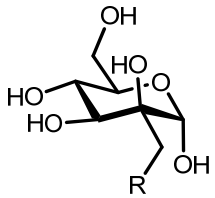


Scheme 1.4 General structures of fucose based ligands developed by Bernardi *et al.*[34]

1.6.1.2 Mannose based

Mannose based DC-SIGN ligands have been studied more extensively than those based on fucose, probably due to the fact that most of the natural DC-SIGN ligands contain mannose or higher mannose structures.

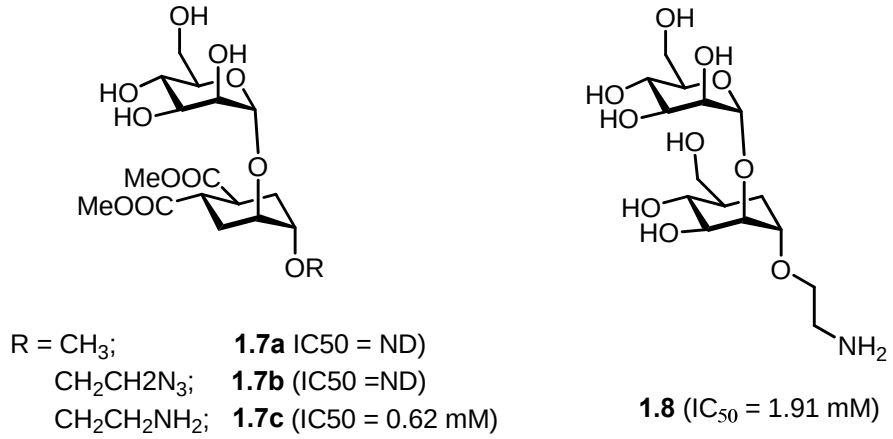
In 2007 a group of 2-C-substituted branched D-mannose analogues **1.6a-d** was reported as better DC-SIGN binder than D-mannose.[36] In particular 2-C-aminomethyl-D-mannose **1.6c** showed 48-fold higher affinity to DC-SIGN (K_i=0.35 mM, K_i(mannose)=17.1 mM), as determined using a surface plasmon resonance (SPR)-based competition assay that measures inhibition of DC-SIGN-HIV gp120 binding (Scheme 1.5).



R = H; **1.6a** (~30% inhibition at 10 mM)
OH; **1.6b** (~50% inhibition at 10 mM)
NH_2; **1.6c** (K_i = 0.35 mM)
N_3; **1.6d** (~70% inhibition at 10 mM)

Scheme 1.5 Branched D-mannose analogues as potent DC-SIGN inhibitor[36]

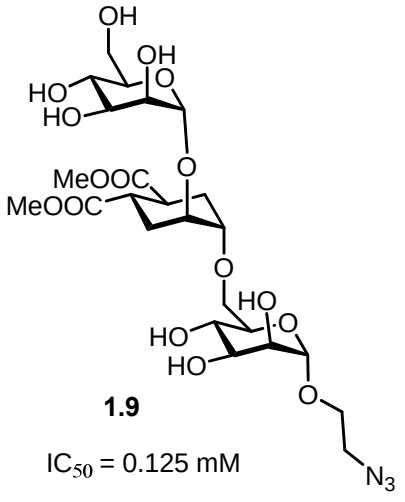
Reina *et al.* published a mimic of the Manα1-2Man dimannoside, where the reducing end is substituted with a conformationally constrained cyclohexane derivative.[37] The molecule is also functionalised either with a methyl (**1.7a**), azidoethyl (**17b**) or aminoethyl (**17c**) group which represents a linker and makes the molecule suitable for multivalent presentations (Scheme 1.6). The activity of these compounds was tested on Ebola virus entry into DC-SIGN expressing Jurkat cells and it was found that compound **1.7c** (IC_{50} = 0.62 mM) is 3 times more active than the natural disaccharide **1.8** (IC_{50} = 1.91 mM).



Scheme 1.6 Structures of the natural dimannoside derivative **1.8** and its mimic **1.7a-c**[37]

STD-NMR of **1.7b** with the extracellular domain of DC-SIGN confirmed that **1.7b** is in close contact with the protein and the mannose mimic at the reducing and also makes interactions with the binding site.

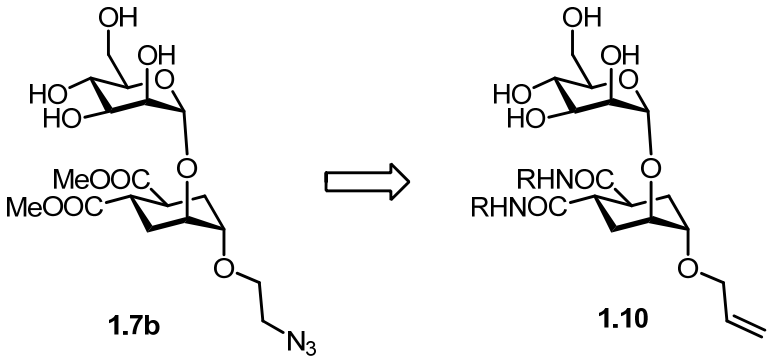
The same research group reported also a trimannoside mimic **1.9**, where the central mannose is replaced with the functionalised cyclohexane used in compounds **1.7a-c** (Scheme 1.7).[38] The prepared DC-SIGN ligands were tested by SPR experiments (competition assay) and it was found that the trimannoside mimic **1.9** is an order of magnitude more active than the corresponding dimannoside **1.7b**.



Scheme 1.7 Pseudo trimannoside derivative **1.9**[38]

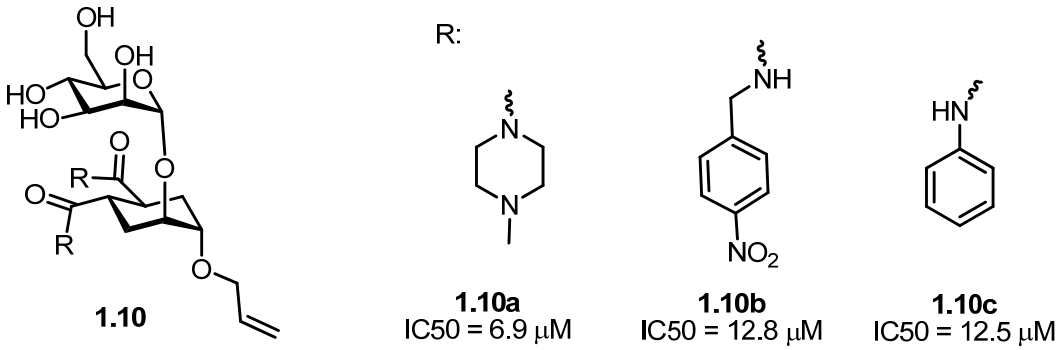
Compound **1.9** showed improvement in activity in comparison with **1.7** but, on the other hand, the trimannoside mimic is synthetically less accessible. In order to gain further interaction with

the binding site the structure of **1.7** was modified by replacement of the methyl esters by amide groups which allowed to introduce lyophilic moieties into the molecule (Scheme 1.8).



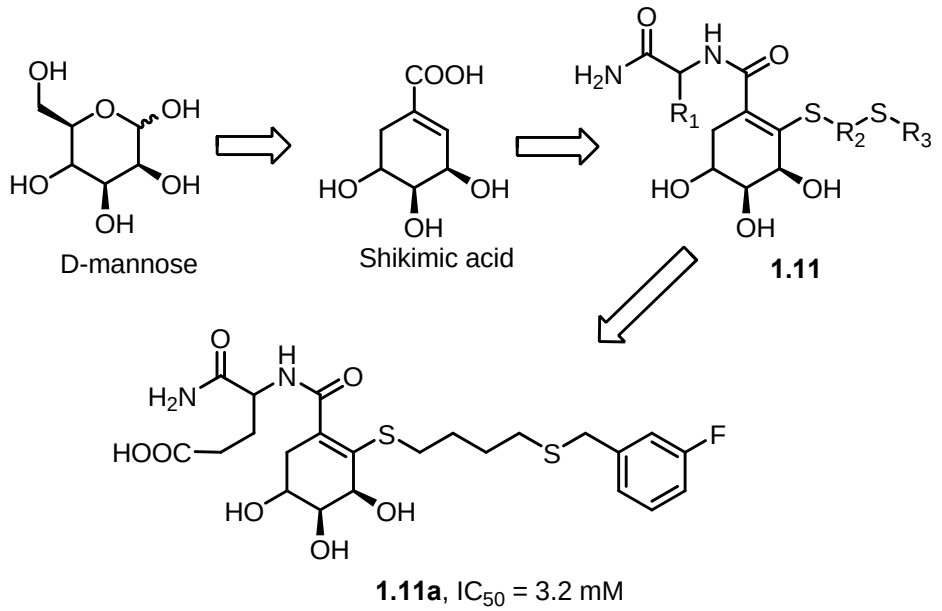
Scheme 1.8 Bisamides **1.10** derived from the dimannoside mimic **1.7c**

A small library of pseudo dimannoside-based bisamides **1.10** was prepared and tested using a DC adhesion assay to mannan-coated plates.[39] It was found that the majority of synthesized compounds inhibit DC adhesion at low micromolar concentrations, which makes them more potent than the starting compound **1.7b** by up to two orders of magnitude (Scheme 1.9).



Scheme 1.9 Bis-amides **1.10a-c** showing IC_{50} values at low micromolar range[39]

Garber et al used Shikimic acid as a mannose mimic since the hydroxyl groups at position 2, 3 and 4 have the same configurations.[40] They designed a DC-SIGN ligand **1.11** based on Shikimic acid and synthesised a library of 192 compounds which were screened using a fluorescence-based, high-throughput competition assay that assesses the ability of compounds to compete with immobilized mannan for binding to the fluorophore labelled DC-SIGN extracellular domain (Scheme 1.10). Compound **1.11a** had the highest affinity with IC_{50} = 3.2 mM while the activity of N-acetylmannosamine was at 11.5 mM.

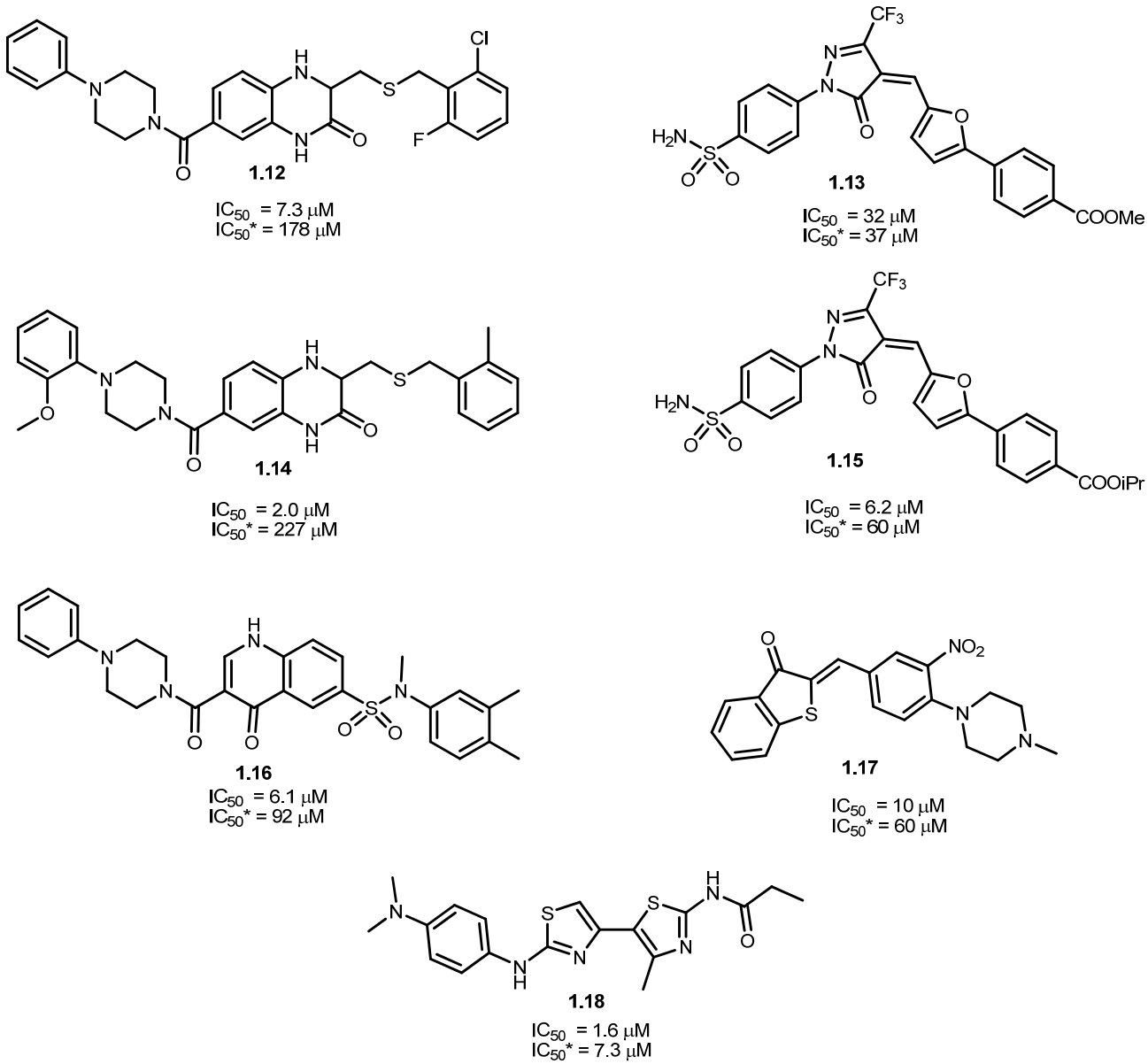


Scheme 1.10 Development of Shikimic acid derived DC-SIGN ligands **1.11**[40]

More importantly, it was found that these compounds are much more selective for DC-SIGN than for mannose binding protein A, which is a C-type lectin found in serum that participates in the innate inflammatory response in defence against a variety of bacterial, fungal and viral pathogens.

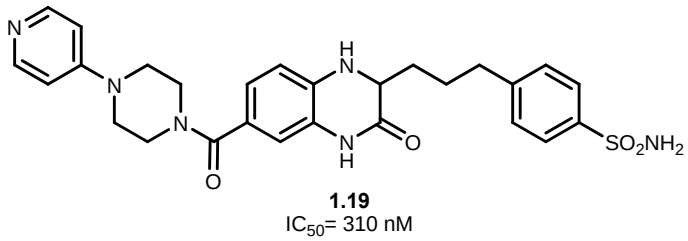
1.6.2 Non-carbohydrate based DC-ligands

Kiessling in a publication from 2007 describes a structurally new class of DC-SIGN ligands.[31] The potential DC-SIGN inhibitors were found after a screening of two libraries of small organic molecules (32 000 compounds). By high throughput screening using immobilised DC-SIGN, 7 compounds **1.12-1.18** were found with activities in the low micromolar range (Scheme 1.11). The molecules have no similarities with native carbohydrates or carbohydrate mimics, proving that sugars are not essential in the design of potential DC-SIGN inhibitors.



Scheme 1.11 Non-carbohydrate based DC-SIGN ligands developed by the group of Kiessling, and their activities determined by the screening assay (IC_{50}) and cell adhesion assays (IC_{50}*)[31]

In a more recent publication from the same group an optimisation of structure **1.12** and **1.14** is described.[32] The most potent ligand **1.19** among the prepared compounds exhibited activity in the nanomolar range (IC_{50} = 310nM) demonstrating that small and highly potent non carbohydrate based DC-SIGN ligands are achievable targets (Scheme 1.12).

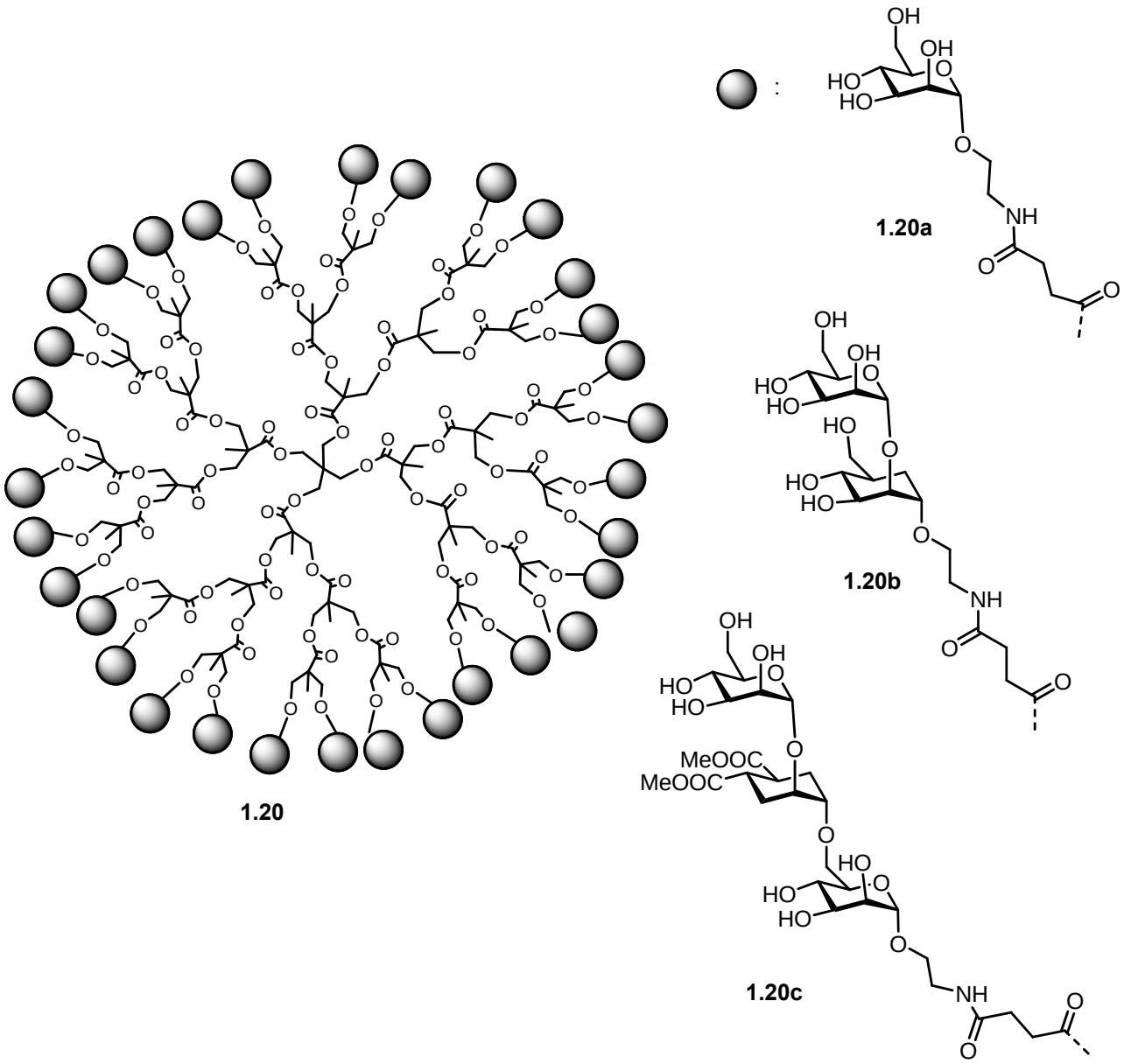


Scheme 1.12 The most potent non-carbohydrate or carbohydrate mimic based DC-SIGN ligand[32]

1.7 Multivalent presentation of carbohydrate based DC-SIGN ligands.

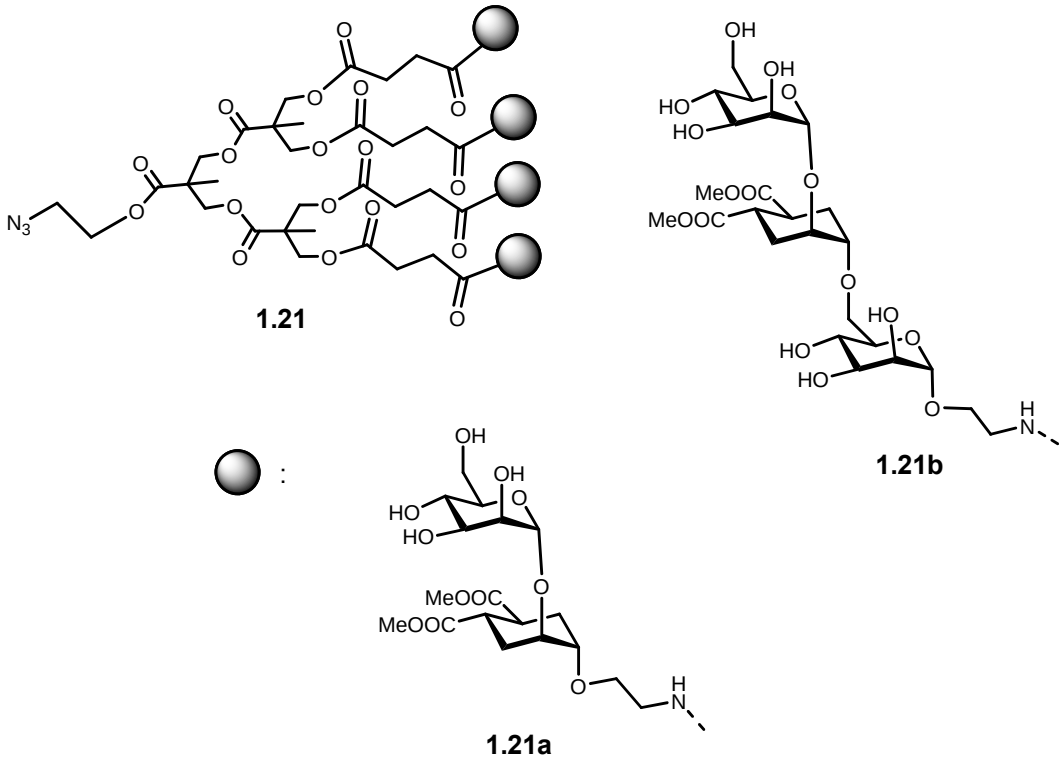
Despite the fact that improvements were achieved in the development of monovalent DC-SIGN ligands, the activities are still too low in comparison with the natural ligands. Nature uses highly glycosylated structures to achieve multivalent interactions with DC-SIGN and this helps significantly to improve its binding activity. This strategy is successively used also in the design of poly or multivalent structures as DC-SIGN ligands.

The first example of using multivalent scaffolds to inhibit DC-SIGN is from the group of Dr. Rojo and Delgado.[41] They used a Boltorn type dendrimer **1.20** functionalised with 32 copies of mannose (**1.20a**) which was tested in *cis* and *trans* DC-SIGN mediated Ebola virus infection studies and the IC50 was found to be in the nanomolar range (IC50 = 337 nm), proving the efficiency of multivalent systems (Scheme 1.13). The pseudo-di and pseudo-trimanmoside mimics **1.7c** and **1.9** were also conjugated to the Boltorn H30 dendrimer (**1.20b, 1.20c**) and tested in cis DC-SIGN mediated Ebola virus infection studies showing activities in low nanomolar range (IC_{50} = 20 nm, Scheme 1.13).[42,43]



Scheme 1.13 Boltorn type dendrimer decorated with mannose-based DC-SIGN inhibitors[41,42,43]

Further, a tetravalent dendron was developed to reduce the loading of the multivalent structures (Scheme 1.14).[43] This dendron was conjugated with **1.17c** and **1.19** and tested in *trans* infection experiments where the IC_{50} value for **1.21a** and **1.21b** was 1.22 μM and 203 nM respectively.

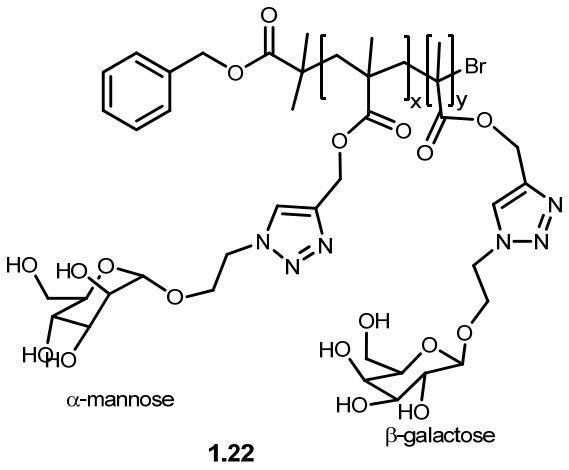


Scheme 1.14 Dendrons bearing sugar mimics **1.17c** and **1.19**[43]

The tetrameric Dendron **1.21** represents a structurally simpler compound in comparison with the 3rd generation Boltorn type dendrimer **1.20**, while the IC_{50} affinity towards DC-SIGN can be still in the nanomolar range.

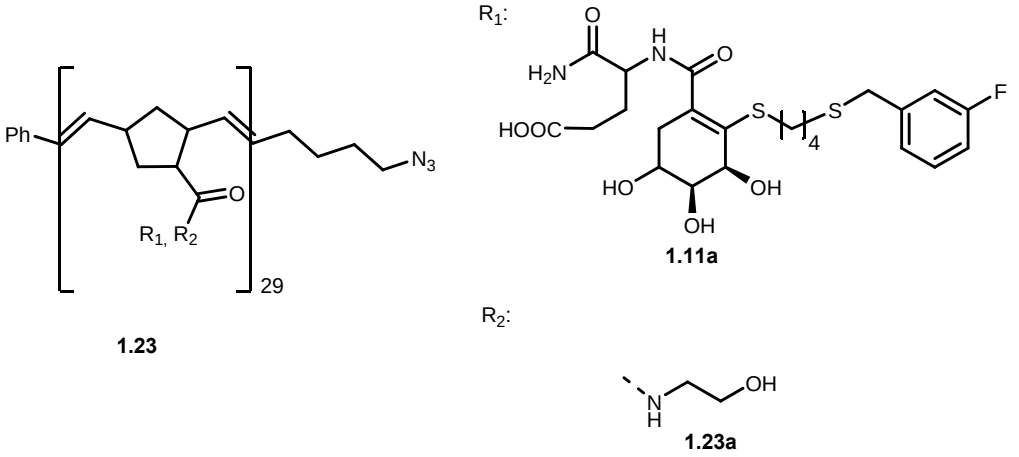
Another type of glycodendron bearing 25 copies of Man4 and Man9 oligosaccharides was developed by Wang *et al.*[46] Using a glycan array assay that measures binding to HIV-1-neutralizing monoclonal antibody and recombinant dimeric DC-SIGN, these glycodendrons exhibited potent inhibition of binding in the low nanomolar range.

Glycopolymers **1.22** functionalised with α-mannose and β-galactose in different ratios have been synthesised by Becer *et al* (Scheme 1.15).[44] The activities of these polymers were determined using an SPR assay that measures inhibition of DC-SIGN-gp120 binding, and it was found that the potency highly depends on the mannose content; an IC_{50} of 37 nM was obtained for a glycopolymer with 100% mannose.



Scheme 1.15 Polymer **1.22** as multivalent scaffold functionalised with α-mannose and β-galactose[44]

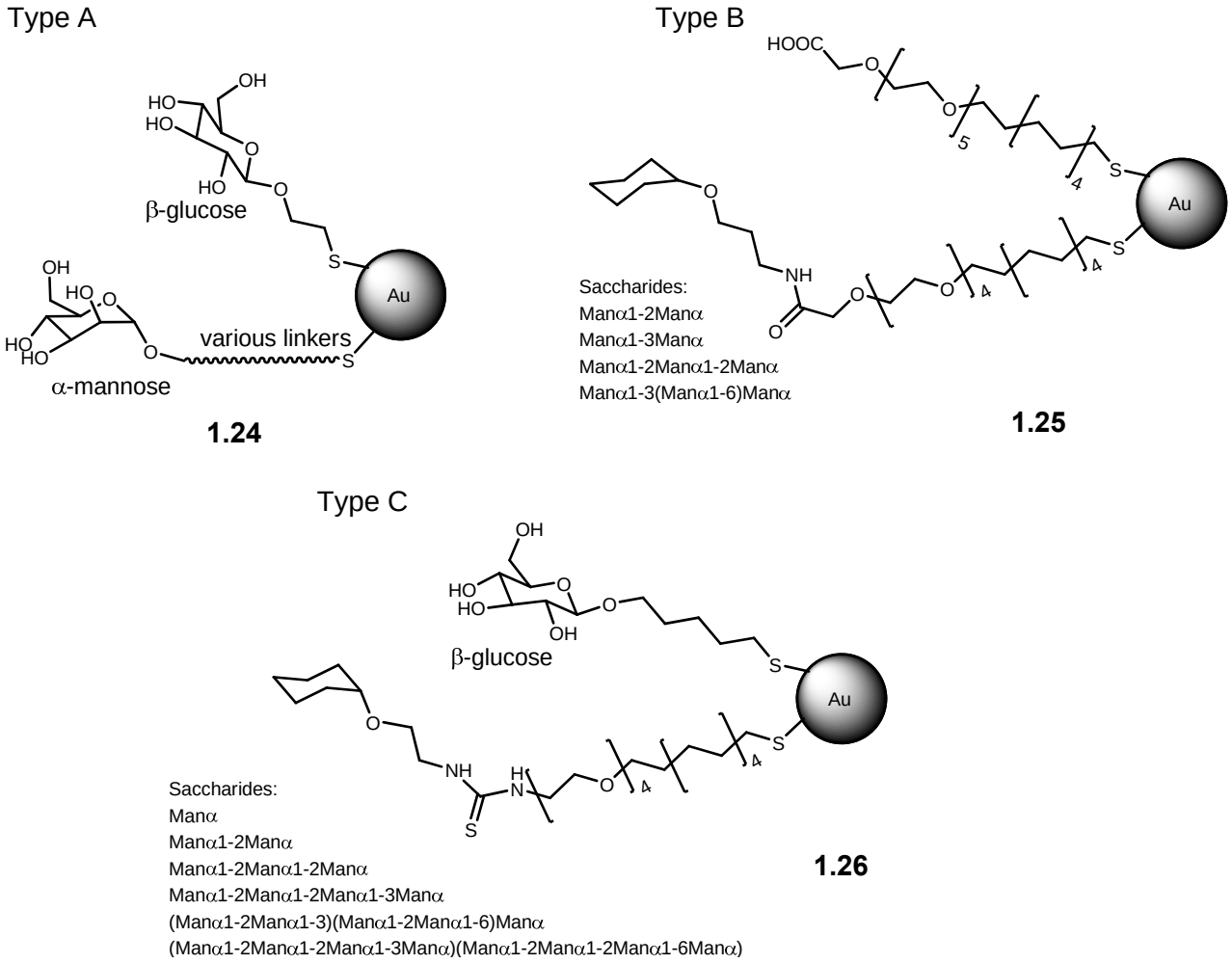
The glycomimetic **1.11a** derived from Shikimic acid was also prepared in multivalent presentation as it was conjugated with a scaffold prepared by ring-opening methathesis (Scheme 1.16).[40] Polymer **1.23** functionalised with 29 copies of **1.11a** has IC_{50} = 2.9 μM and its length should be enough to reach two CRDs of DC-SIGN.



Scheme 1.16 Polymer prepared by ring opening metathesis and functionalised with **1.11a**[40]

Nanoparticles were also used as potential multivalent system to block HIV-1 gp120 binding to DC-SIGN. Martinez-Alvila designed a small library of manno-glyconanoparticles (manno-GNP), where the gold nanoparticles were functionalised with truncated (oligo)mannosides of the high-mannose oligosaccharide ligand for DC-SIGN (undecasaccharide $Man_9GlcNAc_2$, scheme 1.17).[45] Three different types of nanoparticles **1.24**, **1.25** and **1.26** were prepared and tested both in SPR based competition assay and by in vitro assay that measures DC-SIGN mediated HIV-1 *trans*-infection of human T lymphocytes. The results indicate that HIV-1 infection can be successfully inhibited by all GNPs, but the carbohydrate density on the gold surface has a

noticeable effect on the inhibition. In particular, GNP bearing 56 copies of Manα1-2Manα1-2Manα1-3Manα showed remarkable inhibitory potency, with an IC_{50} of 0.34 nM to 0.83 nM, depending on the type of recombinant virus.



Scheme 1.17 Three types of gold nanoparticles bearing different mono and polysaccharides[45]

A significant drawback of gold nanoparticles is their toxicity caused by gold accumulation, however topical use should overcome this problem.

From the previous examples, it is clear that the strategy to obtain potent and selective DC-SIGN antagonists is based on the development of small molecules which are then conjugated to multivalent scaffolds. The effect of multivalency helps significantly to improve the activity of the molecules giving chance for the development of antimicrobial agents which target DC-SIGN.

1.8 References

1. Davis, M. M., Panning for T-cell gold. *Scientist* **2004,** *18* (14), 28-29.

2. Abbas, A. K.; Lichtman, A. H., *Fondamenti di immunologia*. Piccin nuova libreria: 2003.

3. Kapsenberg, M. L., Dendritic-cell control of pathogen-driven T-cell polarization. *Nat Rev Immunol* **2003,** *3* (12), 984-993.

4. van Kooyk, Y.; Geijtenbeek, T. B. H., DC-sign: Escape mechanism for pathogens. *Nat Rev Immunol* **2003,** *3* (9), 697-709.

5. Banchereau, J.; Briere, F.; Caux, C.; Davoust, J.; Lebecque, S.; Liu, Y. T.; Pulendran, B.; Palucka, K., Immunobiology of dendritic cells. *Annu Rev Immunol* **2000,** *18*, 767-+.

6. Svajger, U.; Anderluh, M.; Jeras, M.; Obermajer, N., C-type lectin DC-SIGN: An adhesion, signalling and antigen-uptake molecule that guides dendritic cells in immunity. *Cell Signal* **2010,** *22* (10), 1397-1405.

7. Feinberg, H.; Mitchell, D. A.; Drickamer, K.; Weis, W. I., Structural basis for selective recognition of oligosaccharides by DC-SIGN and DC-SIGNR. *Science* **2001,** *294* (5549), 2163-2166.

8. Geijtenbeek, T. B. H.; van Duijnhoven, G. C. F.; van Vliet, S. J.; Krieger, E.; Vriend, G.; Figdor, C. G.; van Kooyk, Y., Identification of different binding sites in the dendritic cell-specific receptor DC-SIGN for intercellular adhesion molecule 3 and HIV-1. *J Biol Chem* **2002,** *277* (13), 11314-11320.

9. Anderluh, M.; Jug, G.; Svajger, U.; Obermajer, N., DC-SIGN Antagonists, a Potential New Class of Anti-Infectives. *Current medicinal chemistry* **2012,** *19* (7), 992-1007.

10. Mitchell, D. A.; Fadden, A. J.; Drickamer, K., A novel mechanism of carbohydrate recognition by the C-type lectins DC-SIGN and DC-SIGNR - Subunit organization and binding to multivalent ligands. *J Biol Chem* **2001,** *276* (31), 28939-28945.

11. Tabarani, G.; Thepaut, M.; Stroebel, D.; Ebel, C.; Vives, C.; Vachette, P.; Durand, D.; Fieschi, F., DC-SIGN Neck Domain Is a pH-sensor Controlling Oligomerization SAXS and hydrodynamic studies of extracellular domain. *J Biol Chem* **2009,** *284* (32), 21229-21240.

12. Appelmelk, B. J.; van Die, I.; van Vliet, S. J.; Vandenbroucke-Grauls, C. M. J. E.; Geijtenbeek, T. B. H.; van Kooyk, Y., Cutting edge: Carbohydrate profiling identifies new pathogens that interact with dendritic cell-specific ICAM-3-grabbing nonintegrin on dendritic cells. *J Immunol* **2003,** *170* (4), 1635-1639.

13. Tsegaye, T. S.; Pohlmann, S., The multiple facets of HIV attachment to dendritic cell lectins. *Cell Microbiol* **2010,** *12* (11), 1553-1561.

14. Cambi, A.; Beeren, I.; Joosten, B.; Fransen, J. A.; Figdorl, C. G., The C-type lectin DC-SIGN internalizes soluble antigens and HIV-1 virions via a clathrin-dependent mechanism. *Eur J Immunol* **2009,** *39* (7), 1923-1928.

15. Kwon, D. S.; Gregorio, G.; Bitton, N.; Hendrickson, W. A.; Littman, D. R., DC-SIGN-mediated internalization of HIV is required for trans-enhancement of T cell infection. *Immunity* **2002,** *16* (1), 135-144.

16. Geijtenbeek, T. B. H.; Torensma, R.; van Vliet, S. J.; van Duijnhoven, G. C. F.; Adema, G. J.; van Kooyk, Y.; Figdor, C. G., Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. *Cell* **2000,** *100* (5), 575-585.

17. Turville, S.; Wilkinson, J.; Cameron, P.; Dable, J.; Cunningham, A. L., The role of dendritic cell C-type lectin receptors in HIV pathogenesis. *J Leukocyte Biol* **2003,** *74* (5), 710-718.

18. Valladeau, J.; Ravel, O.; Dezutter-Dambuyant, C.; Moore, K.; Kleijmeer, M.; Liu, Y.; Duvert-Frances, V.; Vincent, C.; Schmitt, D.; Davoust, J.; Caux, C.; Lebecque, S.; Saeland, S., Langerin, a novel C-type lectin specific to Langerhans cells, is an endocytic receptor that induces the formation of Birbeck granules. *Immunity* **2000,** *12* (1), 71-81.

19. Feinberg, H.; Powlesland, A. S.; Taylor, M. E.; Weis, W. I., Trimeric Structure of Langerin. *J Biol Chem* **2010,** *285* (17), 13285-13293.

20. Chatwell, L.; Holla, A.; Kaufer, B. B.; Skerra, A., The caxbohydrate recognition domain of Langerin reveals high structural similarity with the one of DC-SIGN but an additional, calcium-independent sugar-binding site. *Mol Immunol* **2008,** *45* (7), 1981-1994.

21. Guo, Y.; Feinberg, H.; Conroy, E.; Mitchell, D. A.; Alvarez, R.; Blixt, O.; Taylor, M. E.; Weis, W. I.; Drickamer, K., Structural basis for distinct ligand-binding and targeting properties of the receptors DC-SIGN and DC-SIGNR. *Nat Struct Mol Biol* **2004,** *11* (7), 591-598.

22. Taylor, M. E.; Drickamer, K., Structural insights into what glycan arrays tell us about how glycan-binding proteins interact with their ligands. *Glycobiology* **2009,** *19* (11), 1155-1162.

23. Adams, E. W.; Ratner, D. M.; Bokesch, H. R.; McMahon, J. B.; O'Keefe, B. R.; Seeberger, P. H., Oligosaccharide and glycoprotein Microarrays as tools in HIV glycobiology: Glycan-dependent gp120/protein interactions. *Chem Biol* **2004,** *11* (6), 875-881.

24. Khoo, U. S.; Chan, K. Y. K.; Chan, V. S. F.; Lin, C. L. S., DC-SIGN and L-SIGN: the SIGNs for infection. *J Mol Med-Jmm* **2008,** *86* (8), 861-874.

25. van Montfort, T.; Eggink, D.; Boot, M.; Tuen, M.; Hioe, C. E.; Berkhout, B.; Sanders, R. W., HIV-1 N-Glycan Composition Governs a Balance between Dendritic Cell-Mediated Viral Transmission and Antigen Presentation. *J Immunol* **2011,** *187* (9), 4676-4685.

26. Sattin, S.; Daghetti, A.; Thepaut, M.; Berzi, A.; Sanchez-Navarro, M.; Tabarani, G.; Rojo, J.; Fieschi, F.; Clerici, M.; Bernardi, A., Inhibition of DC-SIGN-Mediated HIV Infection by a Linear Trimannoside Mimic in a Tetravalent Presentation. *Acs Chem Biol* **2010,** *5* (3), 301-312.

27. Balzarini, J.; Van Herrewege, Y.; Vermeire, K.; Vanham, G.; Schols, D., Carbohydrate-binding agents efficiently prevent dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN)-directed HIV-1 transmission to T lymphocytes. *Mol Pharmacol* **2007,** *71* (1), 3-11.

28. Alen, M. M. F.; Kaptein, S. J. F.; De Burghgraeve, T.; Balzarini, J.; Neyts, J.; Schols, D., Antiviral activity of carbohydrate-binding agents and the role of DC-SIGN in dengue virus infection. *Virology* **2009,** *387* (1), 67-75.

29. Geijtenbeek, T. B. H.; van Vliet, S. J.; Koppel, E. A.; Sanchez-Hernandez, M.; Vandenbroucke-Grauls, C. M. J. E.; Appelmelk, B.; van Kooyk, Y., Mycobacteria target DC-SIGN to suppress dendritic cell function. *J Exp Med* **2003,** *197* (1), 7-17.

30. Mavigner, M.; Cazabat, M.; Dubois, M.; L'Faqihi, F. E.; Requena, M.; Pasquier, C.; Klopp, P.; Amar, J.; Alric, L.; Barange, K.; Vinel, J. P.; Marchou, B.; Massip, P.; Izopet, J.; Delobel, P., Altered CD4(+) T cell homing to the gut impairs mucosal immune reconstitution in treated HIV-infected individuals. *Journal of Clinical Investigation* **2012,** *122* (1), 62-69.

31. Borrok, M. J.; Kiessling, L. L., Non-carbohydrate inhibitors of the lectin DC-SIGN. *J Am Chem Soc* **2007,** *129* (42), 12780-12785.

32. Mangold, S. L.; Prost, L. R.; Kiessling, L. L., Quinoxalinone inhibitors of the lectin DC-SIGN. *Chemical Science* **2012,** *3* (3).

33. (a) Dai, B. B.; Yang, L.; Yang, H. G.; Hu, B. L.; Baltimore, D.; Wang, P., HIV-1 Gag-specific immunity induced by a lentivector-based vaccine directed to dendritic cells. *P Natl Acad Sci USA* **2009,** *106* (48), 20382-20387; (b) Yang, L.; Yang, H.; Rideout, K.; Cho, T.; Il Joo, K.; Ziegler, L.; Elliot, A.; Walls, A.; Yu, D.; Baltimore, D.; Wang, P., Engineered lentivector targeting of dendritic cells for in vivo immunization. *Nat Biotechnol* **2008,** *26* (3), 326-334.

34. Andreini, M.; Doknic, D.; Sutkeviciute, I.; Reina, J. J.; Duan, J. X.; Chabrol, E.; Thepaut, M.; Moroni, E.; Doro, F.; Belvisi, L.; Weiser, J.; Rojo, J.; Fieschi, F.; Bernardi, A., Second generation of fucose-based DC-SIGN ligands: affinity improvement and specificity versus Langerin. *Org Biomol Chem* **2011,** *9* (16), 5778-5786.

35. Timpano, G.; Tabarani, G.; Anderluh, M.; Invernizzi, D.; Vasile, F.; Potenza, D.; Nieto, P. M.; Rojo, J.; Fieschi, F.; Bernardi, A., Synthesis of novel DC-SIGN ligands with an alpha-fucosylamide anchor. *Chembiochem* **2008,** *9* (12), 1921-1930.

36. Mitchell, D. A.; Jones, N. A.; Hunter, S. J.; Cook, J. M. D.; Jenkinson, S. F.; Wormald, M. R.; Dwek, R. A.; Fleet, G. W. J., Synthesis of 2-C-branched derivatives of D-mannose: 2-C-aminomethyl-D-mannose binds to the human C-type lectin DC-SIGN with affinity greater than an order of magnitude compared to that of D-mannose. *Tetrahedron-Asymmetr* **2007,** *18* (12), 1502-1510.

37. Reina, J. J.; Sattin, S.; Invernizzi, D.; Mari, S.; Martinez-Prats, L.; Tabarani, G.; Fieschi, F.; Delgado, R.; Nieto, P. M.; Rojo, J.; Bernardi, A., 1,2-mannobioside mimic: Synthesis, DC-SIGN interaction by NMR and docking, and antiviral activity. *Chemmedchem* **2007,** *2* (7), 1030-1036.

38. Maria, S.; Sanchez-Medina, I.; Mereghetti, P.; Belvisi, L.; Jimenez-Barbero, J.; Bernardi, A., Synthesis and conformational analysis of an alpha-D-mannopyranosyl(1 -> 2)-alpha-D-mannopyranosyl-(1 -> 6)-alpha-D-mannopyranose mimic. *Carbohyd Res* **2007,** *342* (12-13), 1859-1868.

39. Obermajer, N.; Sattin, S.; Colombo, C.; Bruno, M.; Svajger, U.; Anderluh, M.; Bernardi, A., Design, synthesis and activity evaluation of mannose-based DC-SIGN antagonists. *Mol Divers* **2011,** *15* (2), 347-360.

40. Garber, K. C. A.; Wangkanont, K.; Carlson, E. E.; Kiessling, L. L., A general glycomimetic strategy yields non-carbohydrate inhibitors of DC-SIGN. *Chem Commun* **2010,** *46* (36), 6747-6749.

41. Lasala, F.; Arce, E.; Otero, J. R.; Rojo, J.; Delgado, R., Mannosyl glycodendritic structure inhibits DC-SIGN-mediated Ebola virus infection in cis and in trans. *Antimicrob Agents Ch* **2003,** *47* (12), 3970-3972.

42. Bernardi, A.; Cheshev, P., Interfering with the sugar code: Design and synthesis of oligosaccharide mimics. *Chem-Eur J* **2008,** *14* (25), 7434-7441.

43. Luczkowiak, J.; Sattin, S.; Sutkeviciute, I.; Reina, J. J.; Sanchez-Navarro, M.; Thepaut, M.; Martinez-Prats, L.; Daghetti, A.; Fieschi, F.; Delgado, R.; Bernardi, A.; Rojo, J., Pseudosaccharide Functionalized Dendrimers as Potent Inhibitors of DC-SIGN Dependent Ebola Pseudotyped Viral Infection. *Bioconjugate Chem* **2011,** *22* (7), 1354-1365.

44. Becer, C. R.; Gibson, M. I.; Geng, J.; Ilyas, R.; Wallis, R.; Mitchell, D. A.; Haddleton, D. M., High-Affinity Glycopolymer Binding to Human DC-SIGN and Disruption of DC-SIGN Interactions with HIV Envelope Glycoprotein. *J Am Chem Soc* **2010,** *132* (43), 15130-15132.

45. Martinez-Avila, O.; Bedoya, L. M.; Marradi, M.; Clavel, C.; Alcami, J.; Penades, S., Multivalent Manno-Glyconanoparticles Inhibit DC-SIGN-Mediated HIV-1 Trans-infection of Human T Cells. *Chembiochem* **2009,** *10* (11), 1806-1809.

46. Wang, S. K.; Liang, P. H.; Astronomo, R. D.; Hsu, T. L.; Hsieh, S. L.; Burton, D. R.; Wong, C. H., Targeting the carbohydrates on HIV-1: Interaction of oligomannose dendrons with human monoclonal antibody 2G12 and DC-SIGN. *P Natl Acad Sci USA* **2008,** *105* (10), 3690-3695.

Chapter 2

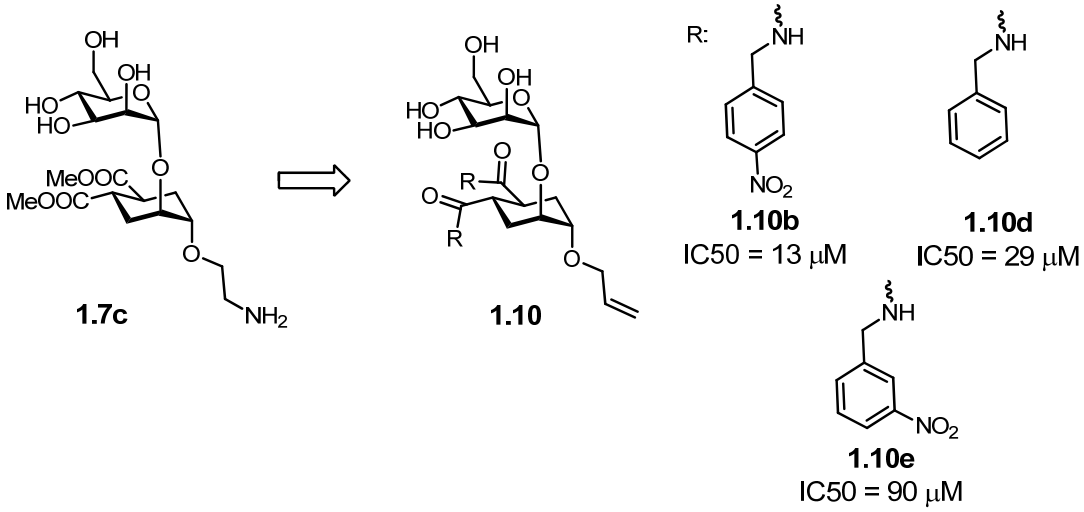
Monovalent glycomimetic DC-SIGN ligands

The synthesis of small organic molecules that bind to DC-SIGN in monovalent fashion is an important step in the development of potent and selective DC-SIGN inhibitors.[1,2,3,4] Compounds mimicking native carbohydrates[5,6,7,8,9] showed promising results to achieve this goal and therefore this project focused mainly on the optimization of the pseudodimannoside (ps-diMan) structures previously developed in the group of professor Anna Bernardi[10,11] in collaboration with the European network CARMUSYS.[12]

The first part of this chapter describes the synthesis of a library of appropriately functionalized ps-diMan based bisamides as well as the activity determination studies that allowed us to select one of the molecules for further elaboration. In the second part of the chapter a modification of the mannose residue in the ps-diMan structure is discussed. A synthetic pathway which allows to introduce a nitrogen atom to the position 6 of this ring is established and SPR measurement show that the modification has positive effect on the activity of some of the prepared ligands.

2.1 Synthesis and activity determination of Pseudodimannoside based bisamides

In a recent publication from the group of Anna Bernardi a library of dimannoside mimics of general formula **1.10** (Scheme 2.1) functionalized with two lipophilic amide groups is described.[11]

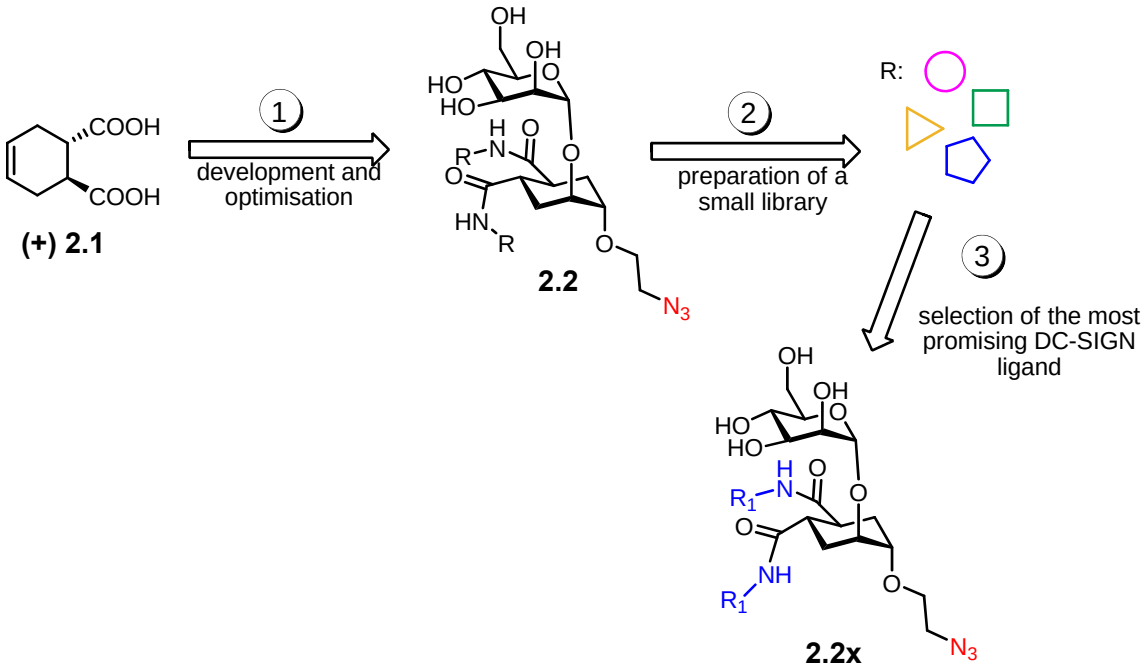


Scheme 2.1 Bisamides **1.10b,d,e** derived from the dimannoside mimic **1.7c** showing IC50 values in the low micromolar range [11]

The prepared bisamides were tested using a DC adhesion assay to mannan-coated plates, and selected compounds were also tested by a SPR technique in which a competition experiment was used. The ligands were tested for their ability to inhibit binding of DC-SIGN to Man-BSA immobilized on the surface of an chip (for details about SPR see section 2.1.4.1). Some of tested

molecules showed improved activity in comparison with the parent methyl ester **1.7c**.[13] These studies allowed to establish that tertiary amides were not effective binders, and that among the molecules studied N-benzyl amides such as **1.10b,d,c** (Scheme 2.1) were the most promising ones. These results were encouraging, since multivalent presentations of the most active bisamides could result in high affinity DC-SIGN ligands. In order to understand the binding mode of the compounds from the **1.10** series with DC-SIGN and to establish what is the contribution of each part of the molecule in the binding process, NMR experiments were performed using STD method (group of professor Pedro Nieto, Seville). The experiments showed relatively high saturation of the allyl function which suggests that this group has non specific interaction with DC-SIGN. This can be reflected in the activity of the compounds as a decrease of the IC50 value which may not correspond to a real antagonistic activity. Moreover, these molecules don't contain a functional group which could be used to connect them with multivalent scaffolds. In order to eliminate the problem regarding the non specific interaction and to obtain a potent monovalent DC-SIGN ligand which can be connected to multivalent scaffolds, three main goals were set for my research (Scheme 2.2):

1. Establish a synthetic pathway for the synthesis of DC-SIGN ligands **2.2** functionalized with an azide-terminated linker which allows conjugation via "click" chemistry (1,3 dipolar cycloaddition)
2. Prepare a small library of ligands and test them in biological assays to evaluate their activities
3. Finally, select the most promising monovalent ligand and synthesize it in large scale for further elaboration towards multivalent systems.



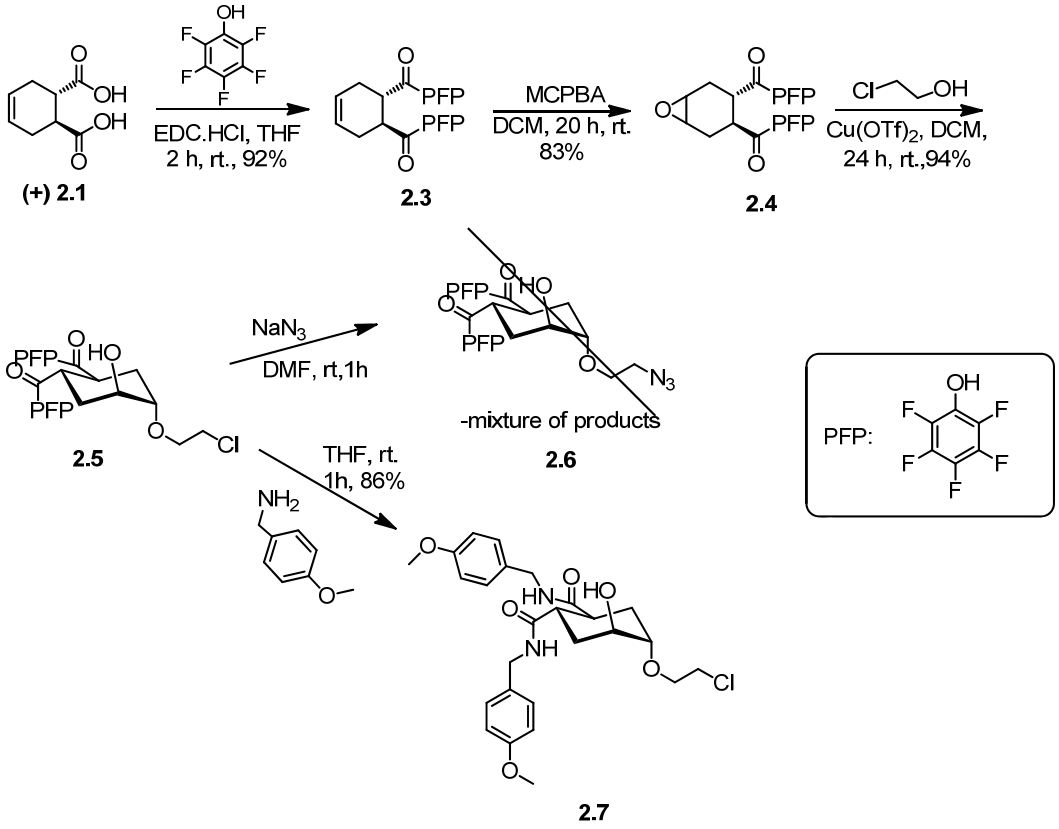
Scheme 2.2 Schematic representation of the strategy used for the development of a potent and selective DC-SIGN inhibitor

2.1.1 Synthesis using pentafluorophenol ester activation

The synthetic approach used for the preparation of compounds **2.2** (Scheme 2.3) is similar to the previously described methodology.[11] It starts from the enantiomerically pure diacid **2.1**.[14] The first step was a di-ester formation with pentafluoro phenol (PFP). This transformation protects the acid in the following two steps, and at the same time provides the required activation of the carboxyl groups for reaction with the amines. The activation was followed by oxidation of the double bond (MCPBA) to afford epoxide **2.4** (Scheme 2.3)**.**

As shown in Scheme 2.3 epoxide **2.4** can be opened with neat chloroethanol using copper(II)triflate (Lewis acid) as a promoter. $Cu/OTf)_2$ was previously selected as the most efficient catalyst in order to open epoxides of these kind.[14] In former procedures epoxide **2.4** (or the methyl ester analogue) was opened with allylic alcohol[11] or 2-bromoethanol.[10] Allylic alcohol allows opening the epoxide with excellent yield (>90%) but unfortunately this linker is not suitable for an easy conjugation to multivalent supports (as mentioned above). Bromoethanol, on the other hand, does not dissolve copper triflate and dichloromethane had to be used as solvent in this reaction, which, in turn, slowed down the process and resulted in low yields (40%) of the corresponding alcohol.[13] Furthermore, purification of the product from the excess of bromoethanol was difficult. Substituting bromoethanol by chloroethanol led to a great improvement of yields (from 40% to 94%) mostly because there is no need of additional solvents

since choroethanol is able to dissolve both the substrate and the promoter.[13] 2-azidoethanol (which was used later, see section 2.1.2) was not considered initially as an option for safety reasons.[15] In summary, during this reaction chloroethanol was used as a nucleophile and solvent to open the epoxide **2.4** in the presence of a catalytic amount of copper(II) triflate. The activated esters are fully stable under these conditions and only one isomer is formed, compound **2.5,** which represents a mannose mimic where the conformationaly constrained cyclohexane[14] is substituted with four functional groups mimicking the α-1,2-mannose configuration.



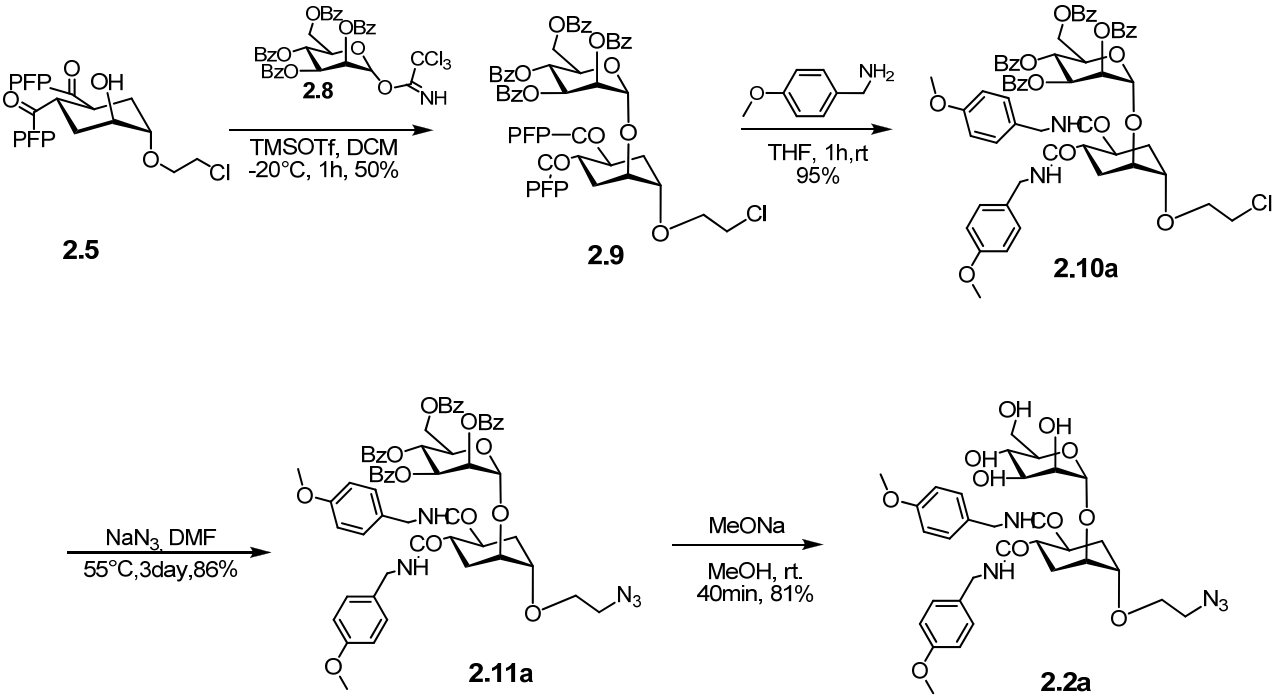
Scheme 2.3 Reactivity determination of the PFP activated ester containing compound **2.5**

An attempt to replace the chloride of **2.5** by an azide using sodium azide in DMF as solvent failed. Within one hour the starting material was consumed and several products were observed in the reaction mixture (TLC). One main product was isolated from the reaction mixture but the NMR signals did not correspond to the desired product **2.6**. Also, the fast reactant consumption indicates that chloride exchange by the azide group did not take place. Most probably the PFP esters are more reactive towards sodium azide than the primary chloride (Scheme 2.3)

The second attempt was the replacement of PFP esters in molecule **2.5** with *p*-methoxybenzyl amine, which was selected as a model amine leading to a potentially active

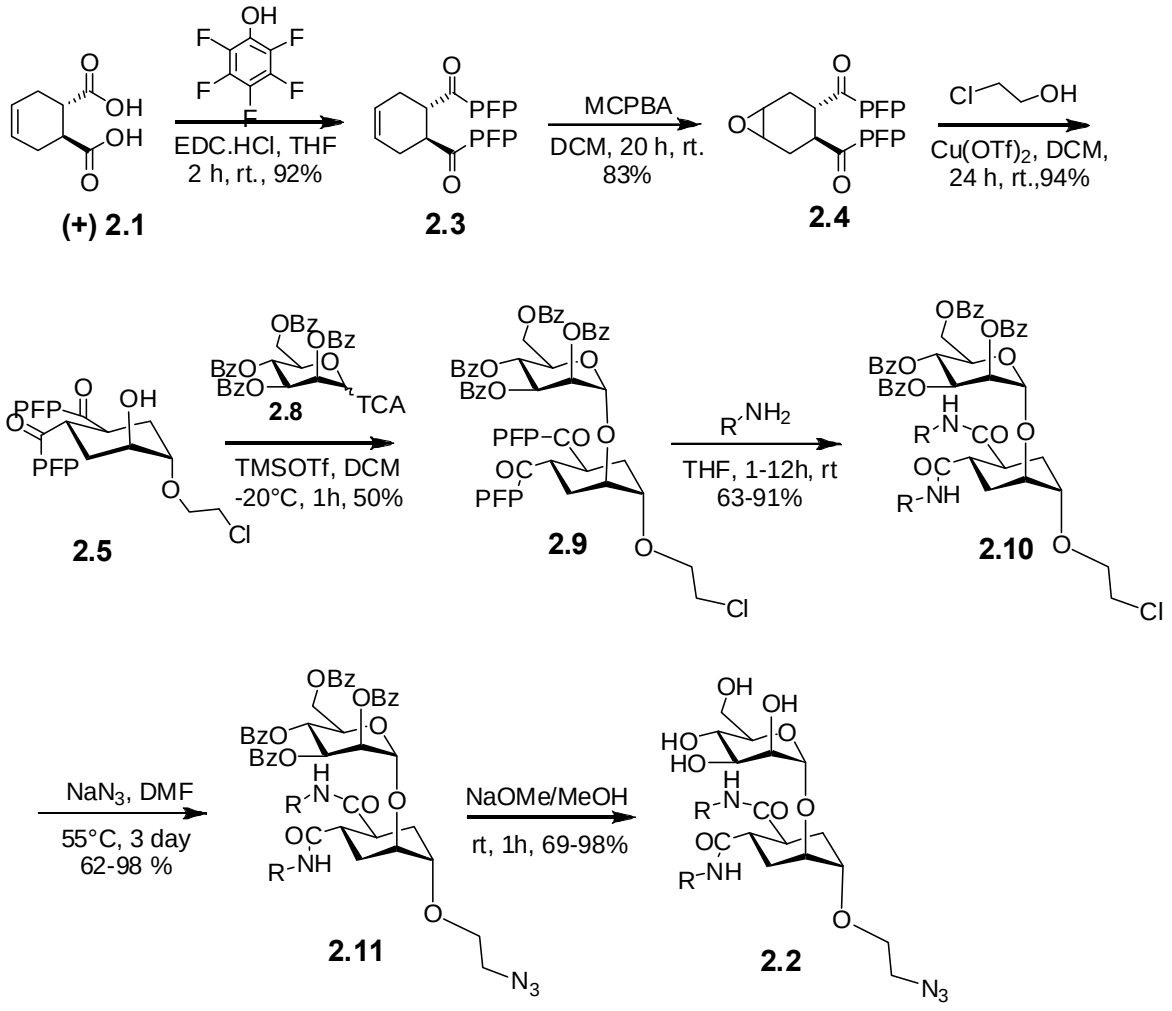
ligand. This reaction worked well, and the primary chloride was not substituted by the amine confirming that the activated esters are indeed more reactive than the chloride (Scheme 2.3)

Having established that the *p*-methoxybenzyl amine reacts chemoselectively with PFP-esters we set to obtain the mannosylated scaffold **2.9** by glycosylation of compound **2.5**. The glycosyl donor in this reaction is mannose **2.8** activated with trichloroacetimidate in the anomeric position and protected by benzoyl groups in all other positions (Scheme 2.4).[16] Trichloroacetimidate (TCA) is a relatively stable functional group and its stability depends on the nature of neighbouring hydroxyl function protecting groups.[17] However, upon a treatment with an acidic catalyst, TCA becomes a powerful leaving group leading to a S_n reaction in the presence of a glycosyl acceptor with a free hydroxyl moiety. Previous studies showed that tetra-O-benzoylmannose-TCA is a better donor than the corresponding tetra-O-acetate giving higher glycosylation yield and reducing the formation of the orthoester byproduct.[13] In this reaction, trimethylsilyltriflate (TMSOTf) was used in catalytic amount as Lewis acid and the product **2.9** was isolated in 50% yield. Then, the exchange of PFP by p-methoxybenzyl amine in molecule **2.9** was made under the same conditions described for **2.7**. Finally, product **2.10a** was treated with sodium azide in DMF to afford **2.11a** in 86% yield. The mannose moiety in compound **2.11a** was deprotected under Zemplen conditions giving the final product **2.2a**, and establishing the full sequence for the synthesis of the first library.



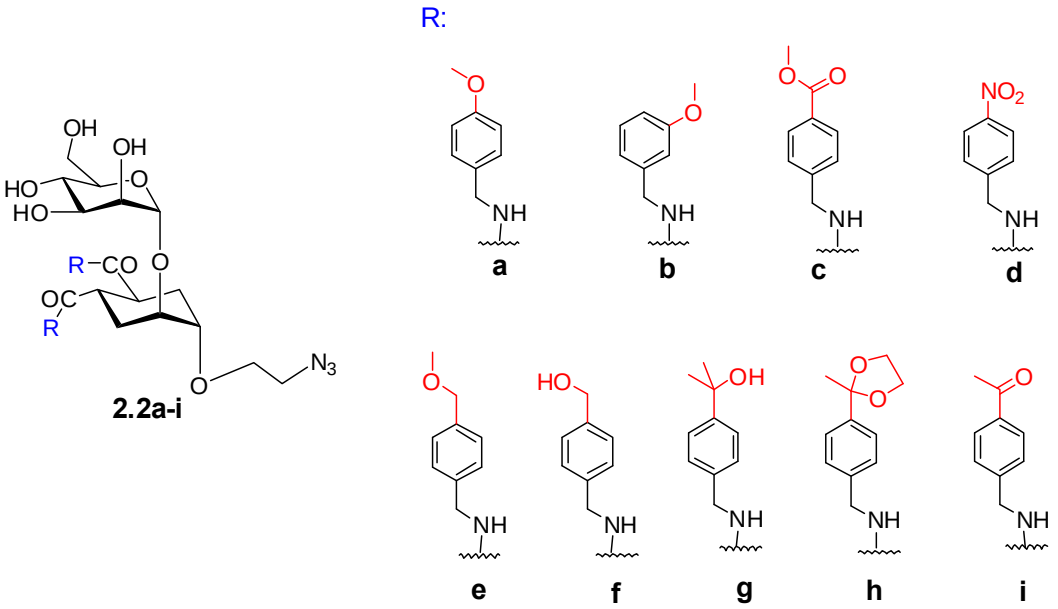
Scheme 2.4 Synthesis of final ligand **2.2a** starting from compound **2.5**

In fact in Scheme 2.4 p-methoxybenzylamine was used as a model nucleophile but this synthetic strategy allows to use different amines. The general synthesis of ligands **2.2** summarizing the reactions from Scheme 2.3 and 2.4 is shown in Scheme 2.5. This strategy was later improved by replacing chloroethanol with azidoethanol, but it served us well for the preparation of the first group of bisamides.



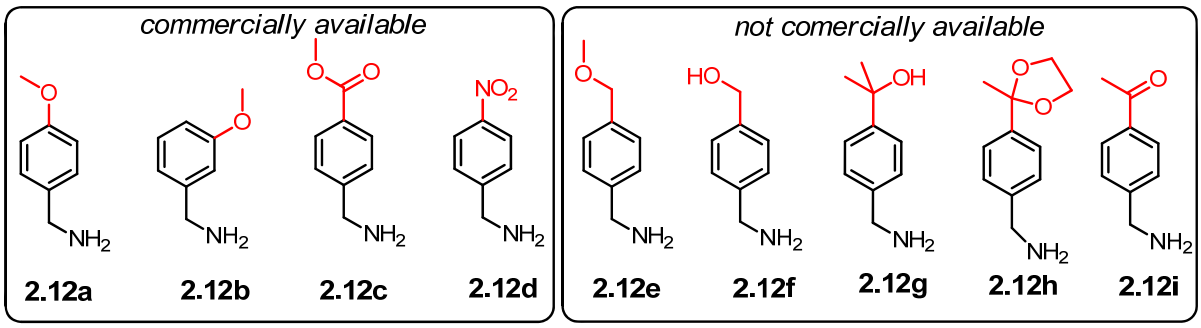
Scheme 2.5 The full synthetic route for the preparation of DC-SIGN ligands **2.2** starting from diacid **2.1**

Among the ligands reported in the previous paper,[11] those with substituted benzyl amides showed high activity (Scheme 2.1). Therefore, a small library of benzylamides substituted with hydrogen bond donor and/or acceptor on the aromatic ring was suggested using docking studies and prepared following the reaction pathway shown in Scheme 2.5. The group of ligands initially suggested is shown in Scheme 2.6.



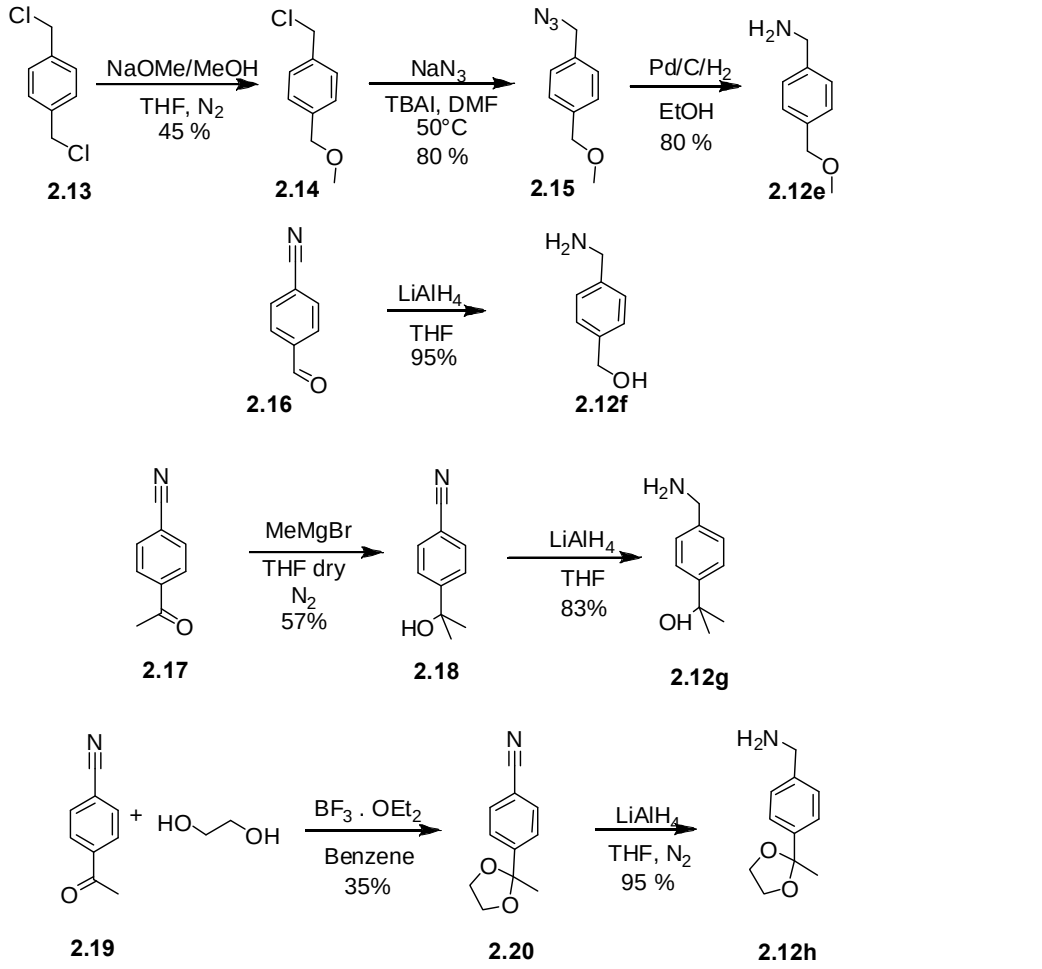
Scheme 2.6 DC-SIGN ligands **2.2a-i** prepared by the method using PFP activation showed in Scheme 2.5

Benzylamines **2.12a-d** used for the synthesis of ligands **2.2a-d** were commercially available (Scheme 2.7) whereas amines **2.12e-i** had to be prepared in 1-3 steps.



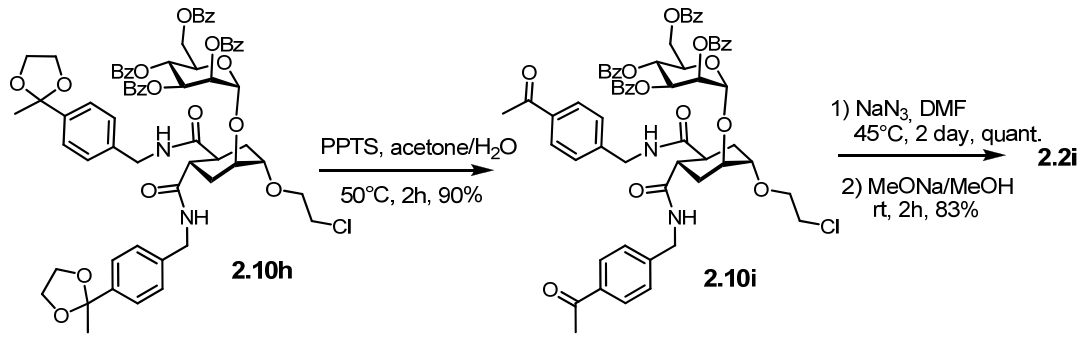
Scheme 2.7 Commercially available and not available benzylamines **2.12a-i** used for the synthesis of DC-SIGN ligands **2.2a-i**

The synthesis of benzyl amines **2.12e-h** used for the preparation of final ligands **2.2e-i** is summarized in Scheme 2.8



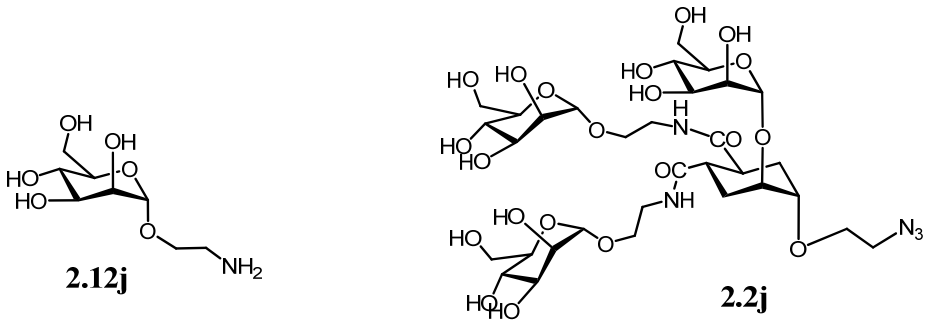
Scheme 2.8 Synthesis of benzylamines **2.12a-h** used for the preparation of final ligands **2.2e-i**

The synthesis of the bis p-acetylbenzylamide derivative **2.2i** required an additional step in comparison with the general procedure shown in Scheme 2.5. The acetal groups in compound **2.10h** (intermediate during the preparation of **2.2h**) were hydrolyzed using a catalytic amount of pyridinium 4-toluenesulfonate (PPTS) leading to compound **2.10i** (Scheme 2.9)**.** The last two steps (exchange of chloride by an azide and deprotection) were identical to those showed in Scheme 2.5.



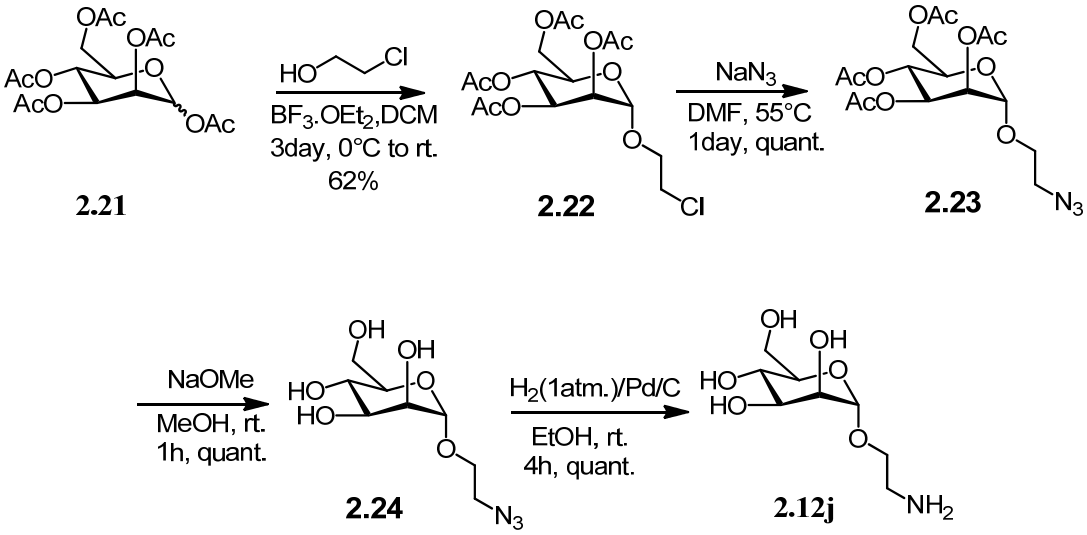
Scheme 2.9 Synthesis of final ligand **2.2i** starting from compound **2.10h**

In an effort to establish if polymannosylation of the scaffold could be beneficial for DC-SIGN binding, also sugar moieties were introduced into the pseudo-disaccharide scaffold through amide bonds. An α-O-mannosyl ethanolamine was used as the carbohydrate residue (Scheme 2.10).



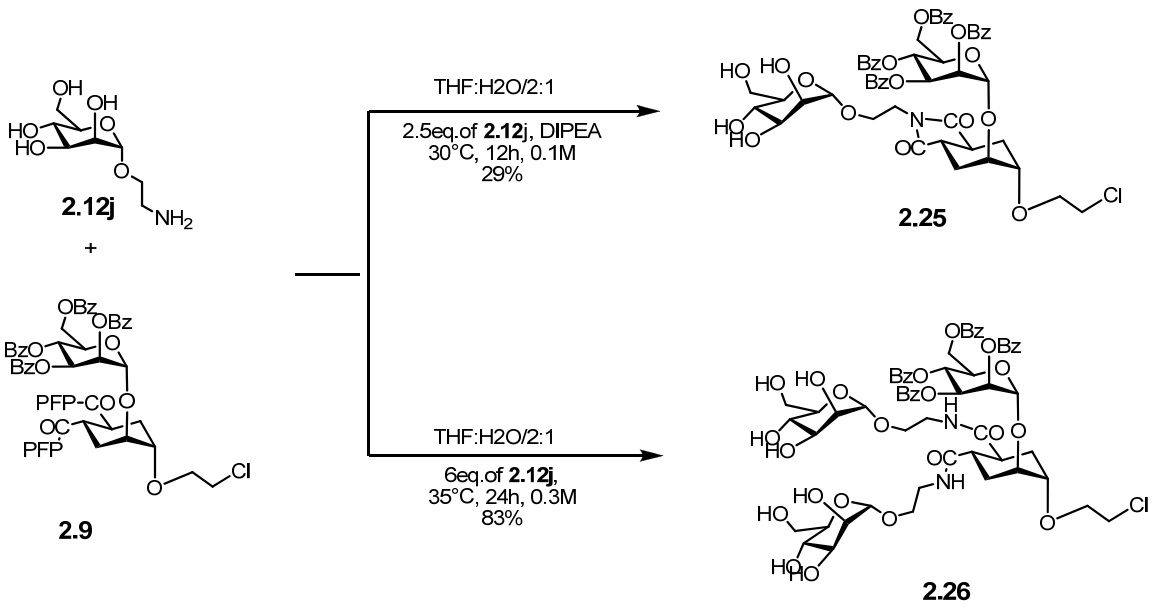
Scheme 2.10 Structure of mannose derivative **2.12j** used for the synthesis of ligand **2.2j**

The synthesis of **2.2j** started with the preparation of α-O-mannosyl ethanolamine **2.12j** (Scheme 2.11) following an established protocol.[18,19] The first step is a reaction between penta-O-acetyl mannose **2.21** and 2-chloroethanol, in the presence of excess $BF_3.OEt_2$. The resulting compound **2.22** was treated with NaN_3 in DMF in order to substitute the chloride by an azide group. The azide derivative **2.23** was then deprotected using sodium methoxide. The last step was the reduction of the azide **2.24** to the corresponding amine **2.12j** (Pd/C, quant).



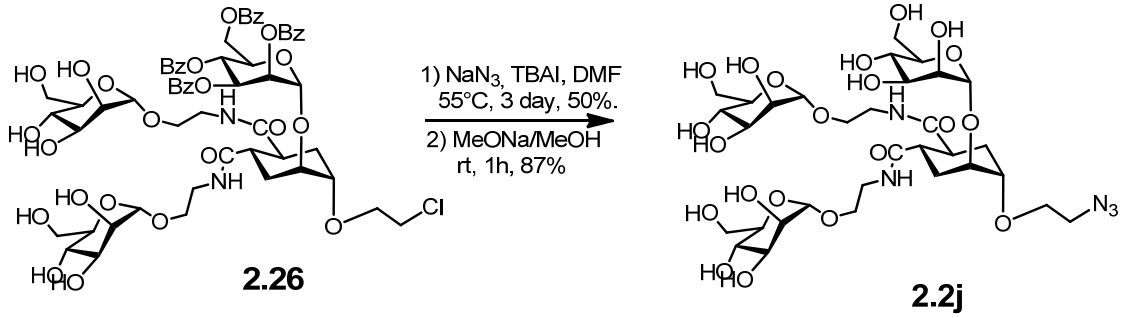
Scheme 2.11 Synthesis of mannose derivative **2.12j**

Condensation of the O-mannosyl ethanolamine **2.12j** with the PFP-activated scaffold **2.9** was not successful when 2.5 molar equivalents of amine **2.12j**, diisopropylethylamine (DIPEA) and 0.1 M concentration of scaffold **2.9** in THF/water mixture was used. Under these conditions only the undesired imide **2.25** was isolated (Scheme 2.12). However, if the amount of amine **2.12j** was increased to 6 molar equivalents, no DIPEA was used and the concentration of **2.9** was 0.3M, the desired bis amide **2.26** was isolated from the reaction mixture. Slow addition (8h) of a solution of **2.9** to the concentrated solution of **2.12j** afforded **2.26** in 82% yield**.**



Scheme 2.12 Reaction condition optimization for the synthesis of **2.32**

Compound **2.26**, after chloride to azide transformation and deprotection, gave the final product **2.2j** (Scheme 2.13).

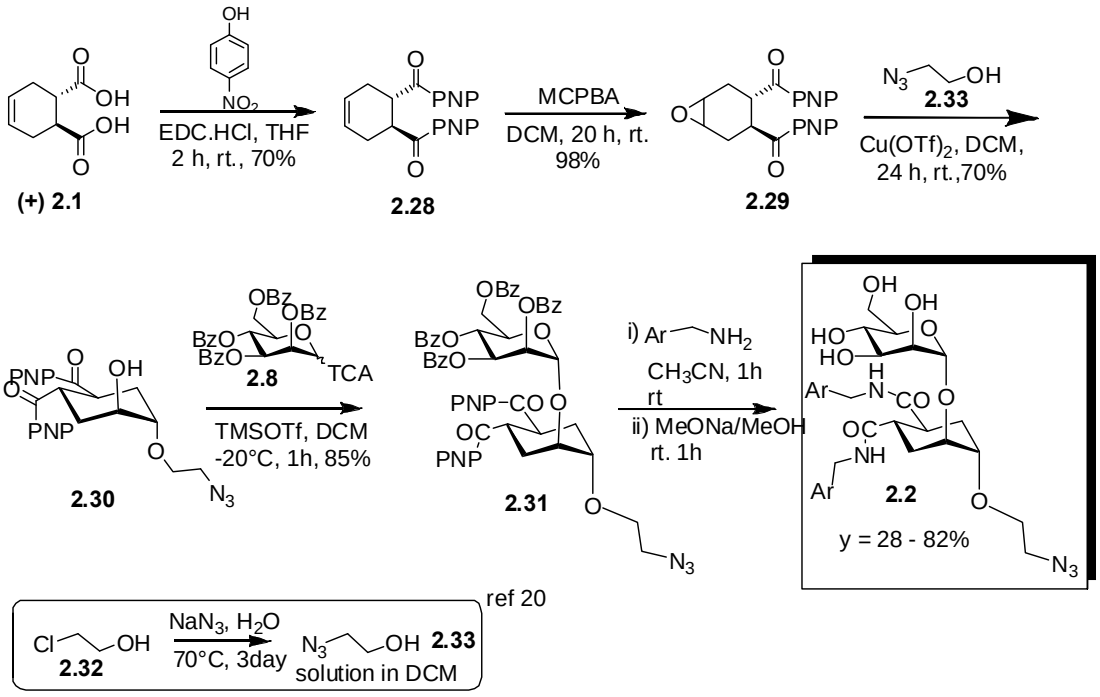


Scheme 2.13 Transformation of intermediate **2.26** to the final ligand **2.2j**

2.1.2 Optimized synthesis of the bisamide ligands

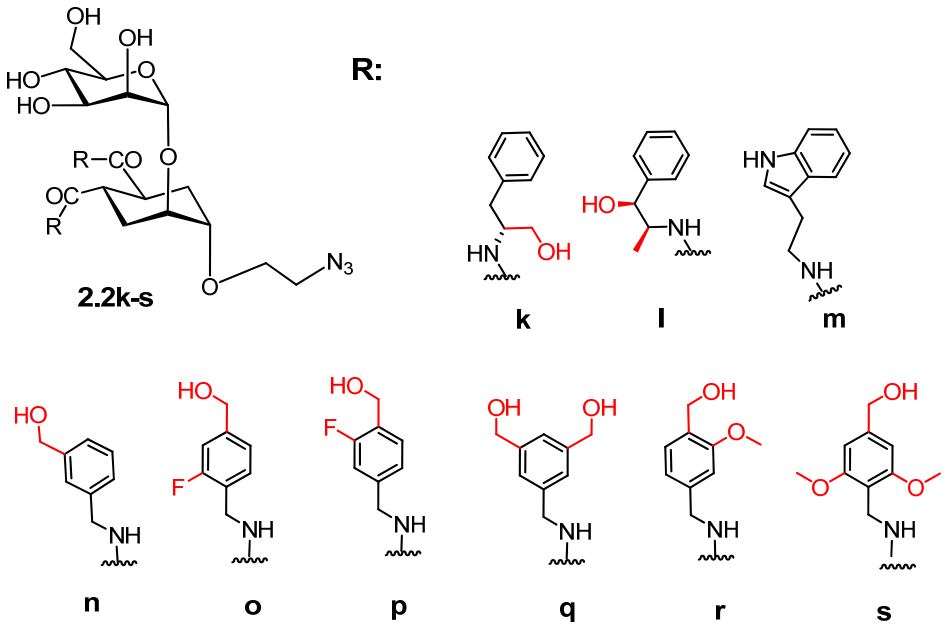
The reaction route shown in the previous section has some drawbacks, most notably: a) the low reaction yield in the glycosylation step of the pentafluorophenyl ester derivative **2.5**, b) the nucleophilic substitution of the Cl atom in **2.10** by NaN_3, which must be performed after the transformation of the activated esters and therefore individually for each final derivative **2.2** from building block **2.9**, and c) the relatively high prize of pentafluorophenol.

For these reasons, a new sequence was suggested which involves p-nitrophenol, as a cheaper activating ester in comparison with the pentafluorophenol ester (Scheme 2.14). The first two steps in the reaction sequence, the activated ester formation and epoxidation, were analogous to those performed with pentafluorophenol. In the following step 2-azidoethanol **2.33**[20] was used to open the epoxide **2.29** (Scheme 2.14). Azidoethanol can be prepared via a reaction in which 2-cloroethanol **2.32** is treated with sodium azide in water media and the resulting reaction mixture is extracted with DCM. The product is not dried due to the volatility and possible explosive properties of **2.33**.[15] Azide derivative **2.33** was prepared several times in small scale with attention on safety issues, and no hazardeous character was observed. The reaction between epoxide **2.29** and alcohol **2.33** resulted in the important intermediate **2.30**, with the activated ester still on, and the azide-terminated linker already installed in the molecule. This compound was glycosylated using trichloroacetimidate activated and benzoyl protected mannose,[16] using the same conditions described in section 2.1.1, and resulting in building block **2.31** which is a common starting material for all final molecules **2.2**. The last two steps in the synthesis are the treatment of scaffold **2.31** with the corresponding amine in acetonitrile and the subsequent deprotection of the mannose moiety. It was found that these two reactions can be performed in one pot (see general procedure 5 in the experimental part) and thus accelerating and simplifying the synthesis of the final ligands (Scheme 2.14).



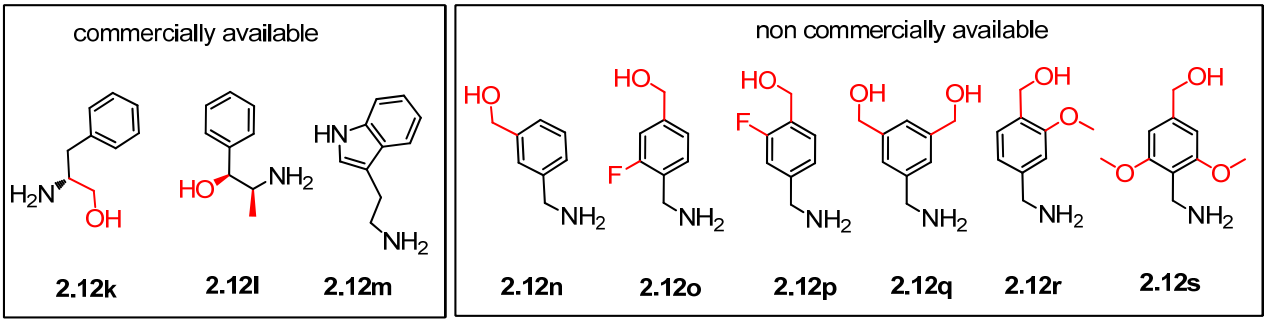
Scheme 2.14 Improved synthesis of compounds **2.2** using PNP activation

The first set of ligands **2.2a-j**, described in the previous section, was tested by single point SPR experiments (discussed in details in section 2.1.4.2) in which compound **2.2f** was found to be the most interesting one for further modifications. For this reason, another small focused library of molecules was designed and prepared (using the optimized synthesis) including derivatives of compound **2.2f** as well as some ligands with different aromatic groups (Scheme 2.15).



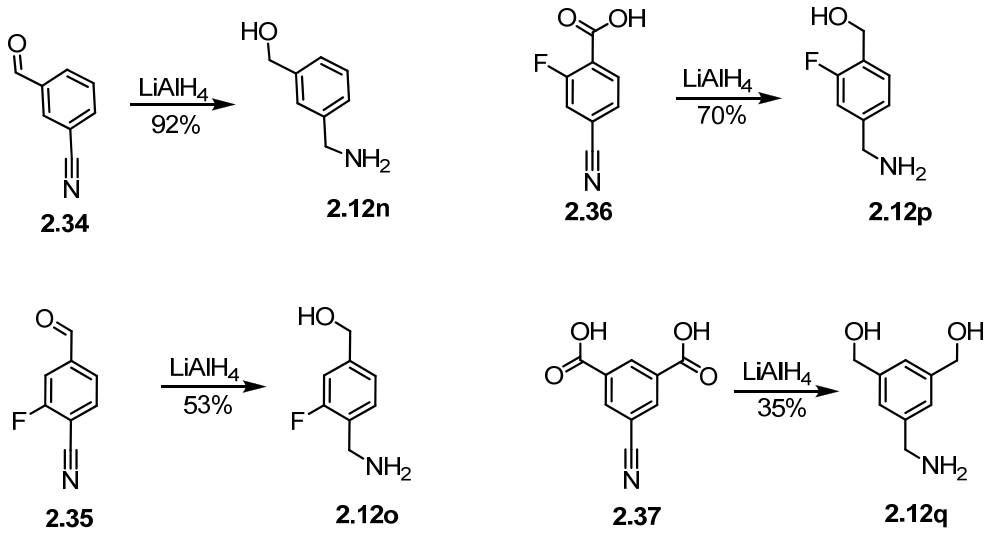
Scheme 2.15 Ligands **2.2k-s** prepared by the method showed in scheme 2.14

Aromatic amines used for the synthesis of **2.2k-m** were commercially available (Scheme 2.16).



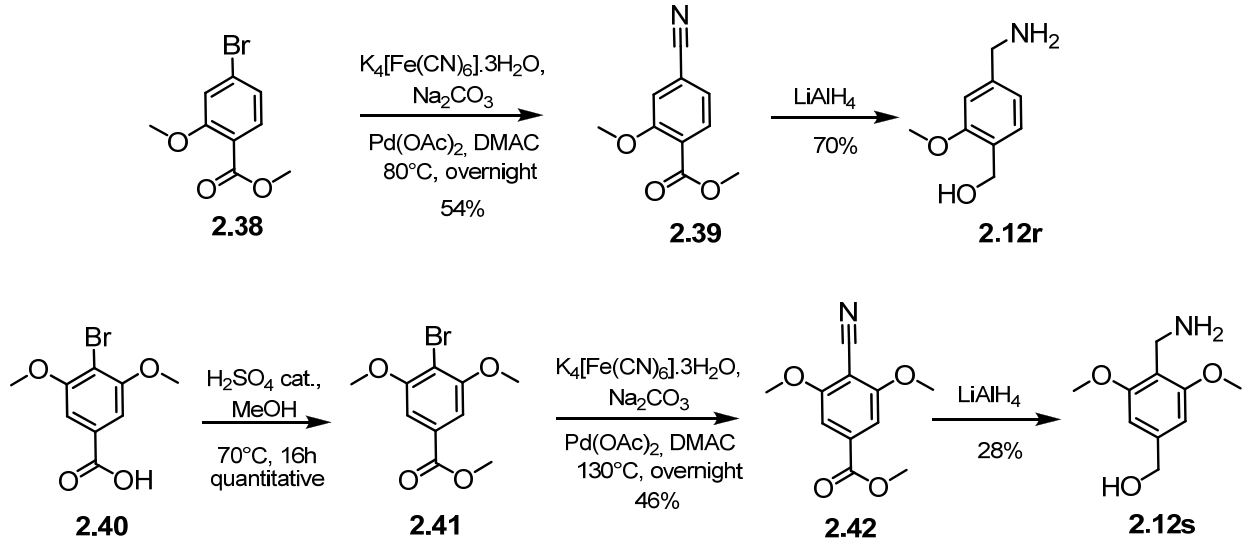
Scheme 2.16 Amines **2.12k-s** used for the synthesis of ligands **2.2k-s**

Amines **2.12n-q** used for the synthesis of **2.2n-q** can be prepared from commercially available materials using strong reductive conditions (Scheme 2.17, see general procedure 1 in the experimental part).



Scheme 2.17 Synthesis of benzylamines **2.12n-q** by reduction with $LiAlH_4$

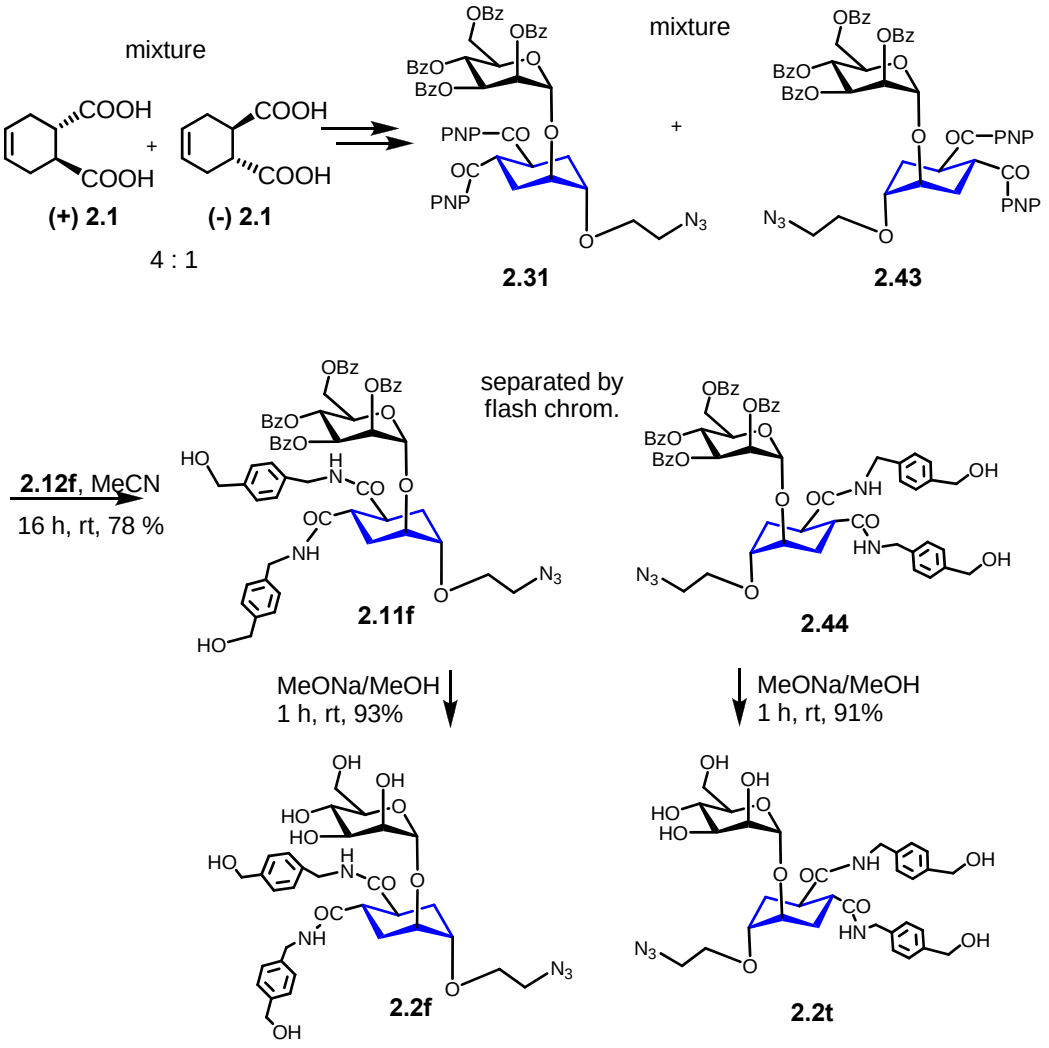
Amines **2.12r-s** used for the preparation of compounds **2.2r-s** were prepared in multistep synthesis. The key step in the following reactions is the palladium catalyzed bromide substitution by a cyanide group.[21] In this reaction $K_4[Fe(CN)_6]$. $3H_2O$ as a non toxic cyanide source was used in the presence of sodium carbonate and catalytic amount of $Pd(OAc)_2$. As solvent dimethylacetamide (DMAC) was used which allows to perform the reactions at high temperatures.



Scheme 2.18 Synthesis of benzylamines using multistep synthesis

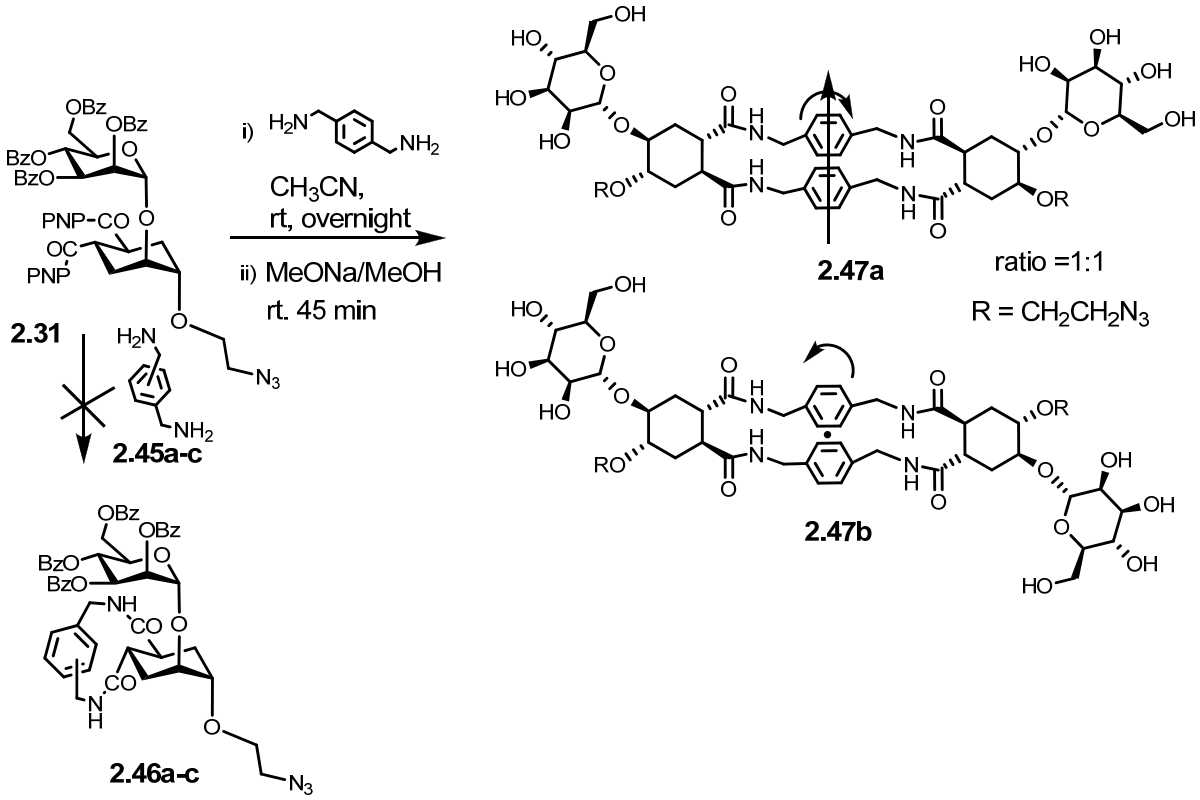
Compound **2.39** was obtained in good yield using only 0.5% of $Pd(OAc)_4$ at 80°C. The synthesis of **2.12s** started from aromatic derivative **2.40**. The acid in compound **2.40** was converted to an ester since there are no examples of carboxylic acids as substrates in the article describing the palladium catalyzed bromide substitution.[21] The subsequent introduction of the cyanide group in **2.41** required higher amount of palladium catalyst (5%) and elevated temperature (130°) but the product **2.42** was obtained in a remarkable yield despite the significant sterical hindrance caused by the bulky methoxy substituents in the *ortho* positions. The last reaction for both **2.39** and **2.42** is the reduction of ester and cyanide functions using $LiAlH_4$ (Scheme 2.18).

To analyze the effect of the stereochemistry of the cyclohexane scaffold on the activity of ligands **2.2,** a stereoisomer of **2.2f,** compound **2.2t** was also prepared. During a large scale synthesis of **2.2f,** the diacid **2.1** was used as a 4:1 mixture of the two enantiomers (commercially available, the resolution of **(+)2.1** is described in the experimental part, section 2.4.2.1). Following the reaction path described in Scheme 2.14 an approximately 4:1 mixture of diastereoisomers **2.31** and **2.43** was obtained and treated with amine **2.12f** (Scheme 2.19). This led to two diastereoisomers **2.11f** and **2.44** which were partially separable by flash chromatography. Deprotection of these compounds gave the final DC-SIGN ligands **2.2f** and its diastereoisomer **2.2t,** respectively (Scheme 2.19).



Scheme 2.19 Schematic representation of the synthesis of diastereoisomers **2.2f** and **2.2t**

In order to investigate the scope of the amide synthesis we also examined the reaction of **2.31** with bis-bezylamines **2.45a-c** (Scheme 2.20). The working hypothesis was that compounds with general structure **2.46** could be obtained. The initial approach using *orto*, *meta* or *para* xylylenediamine **2.45a-c** in the reaction with **2.31** led mostly to complex mixtures. However, in the case of *p*-xylilendiamine one major product could be isolated in moderate yield by chromatography. MS and ^{13}C-NMR analysis revealed that the dimeric macrocyclic structure **2.47** had been formed, as a 1:1 mixture of regioisomers **2.47a** and **2.47b** that could not be separated by chromatographic methods.



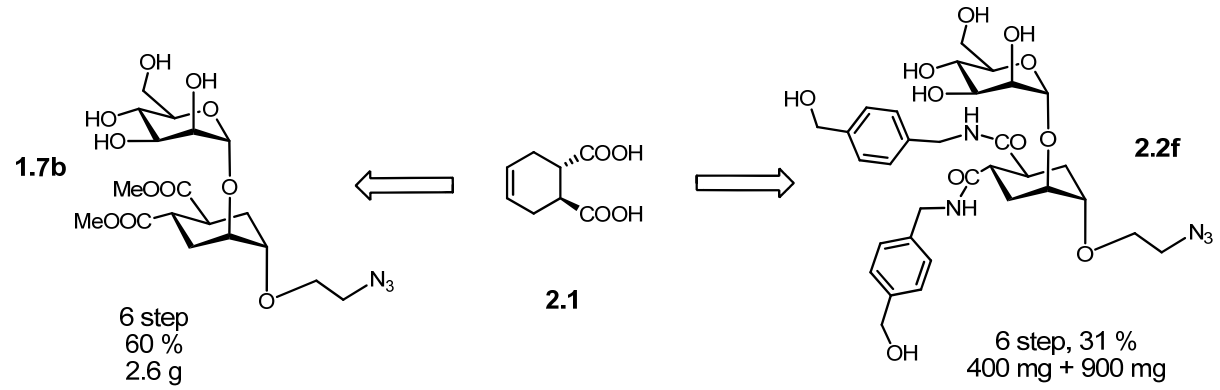
Scheme 2.20 Synthesis of macrocycles **2.47a** and **b**

Sugar containing macrocycles are synthetically challenging structures,[22,23] and this approach gives a relatively easy access to the preparation of this kind of molecules. Compound **2.47** represents a bivalent presentation of DC-SIGN ligands **2.2** and the potential metal chelating properties[24] of the macrocyclic structure could be a target of further investigations.

2.1.3 Large scale synthesis of 1.7b and 2.2f

It was found during my thesis that compounds **1.7b** and **2.2f** represent pseudo dimannose derivatives with good DC-SIGN inhibition activity and therefore these compounds are currently used as standards in biological assays throughout the CARMUSYS network. As it will be described in the following chapter, ligands **1.7b** and **2.2f** were also used in the synthesis of multivalent systems meaning that these compounds had to be available in a relatively large scale. The small scale synthesis of molecule **1.7b** was optimized by Sara Sattin;[13] the scaled up synthesis works just as well, with similar yields. From diacid **2.1** the final compound was obtained in 6 steps with 60% over all yield**.**

Ligand **2.2f** was prepared twice in hundred milligram scales.. Starting from the diacid **2.1** **i**n 6 steps the final compound was obtained in 31% overall yield (Scheme 2.21).



Scheme 2.21 Large scale synthesis of DC-SIGN ligands **1.7b** and **2.2f** both prepared from the enantiomerically pure diacid **2.**

2.1.4 Activity determinations

2.1.4.1 Surface Plasmon Resonance (SPR)

Binding studies on the isolated receptor DC-SIGN, exploiting the SPR (Surface Plasmon Resonance) biosensor, allowed to assess the affinity of the glycomimetic structures. Biosensors used in drug discovery usually require at some point labeling (fluorescent or radiolabelling) of at least one of the components involved in the interaction process to report the binding of a ligand to its receptor. This labeling step demands extra time and cost, and can, in some cases lead to false negatives or false positives. SPR, as well as other optical biosensors, exploits the evanescent-wave phenomenon to characterize interactions between receptors that are usually attached to the biosensor surface and ligands that are in solution above the surface. The response signal is directly proportional to the amount in weight of bound ligand. In figure 2.1 is a schematic view of a SPR biosensor.

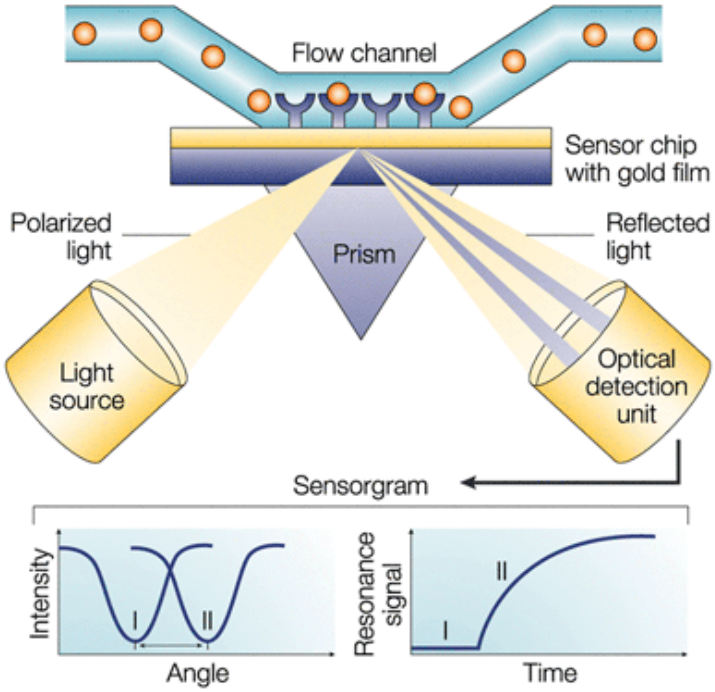


Figure 2.1 Schematic representation of the principles of SPR [25]

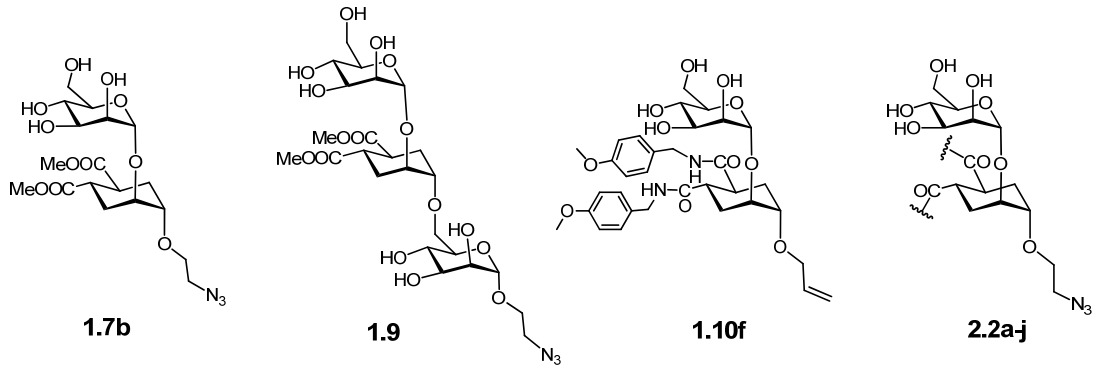
Surface plasmon resonance (SPR) detects changes in the refractive index in the immediate vicinity of the surface layer of a sensor chip. SPR is observed as a sharp shadow in the reflected light from the surface at an angle that is dependent on the mass of material at the surface. The SPR angle shifts (from I to II in the lower left-hand diagram) when (bio)molecules bind to the surface and change the mass of the surface layer.[25]

There are several different possible experimental set up, depending on the molecules we are handling, where the immobilized molecule can be the ligand, the receptor or a binding competitor. Low molecular weight ligands (as in the case of monovalent carbohydrates binding to a lectin) are difficult to detect in general, and therefore we chose to immobilize the binding competitor. In our particular case, a highly mannosylated glycoprotein was immobilized on the SPR chip and a fixed concentration of DC-SIGN extra-cellular domain (ECD) was flowed within a) increasing concentration of the tested ligand in order to determine its IC50 value, or b) fixed concentration of the ligand in the case of single point SPR experiment when the inhibition potency of the ligand is measured at certain (fixed) concentration. More in detail, for this assay, a CM4 SPR chip was used. Two flow cells were activated as previously described[26] with an EDC-NHS mixture. Flow cell one was then blocked with 1M ethanolamine (50 µL) and served as a control surface. The second one was treated with Man-BSA (Bovine serum albumin - Mannotriose, Dextra, 60 µg/mL) in acetate buffer (10 mm, pH 4). The Man-BSA used to functionalize the CM4 chips harbors 15 glycosylation sites (according to the manufacturer)

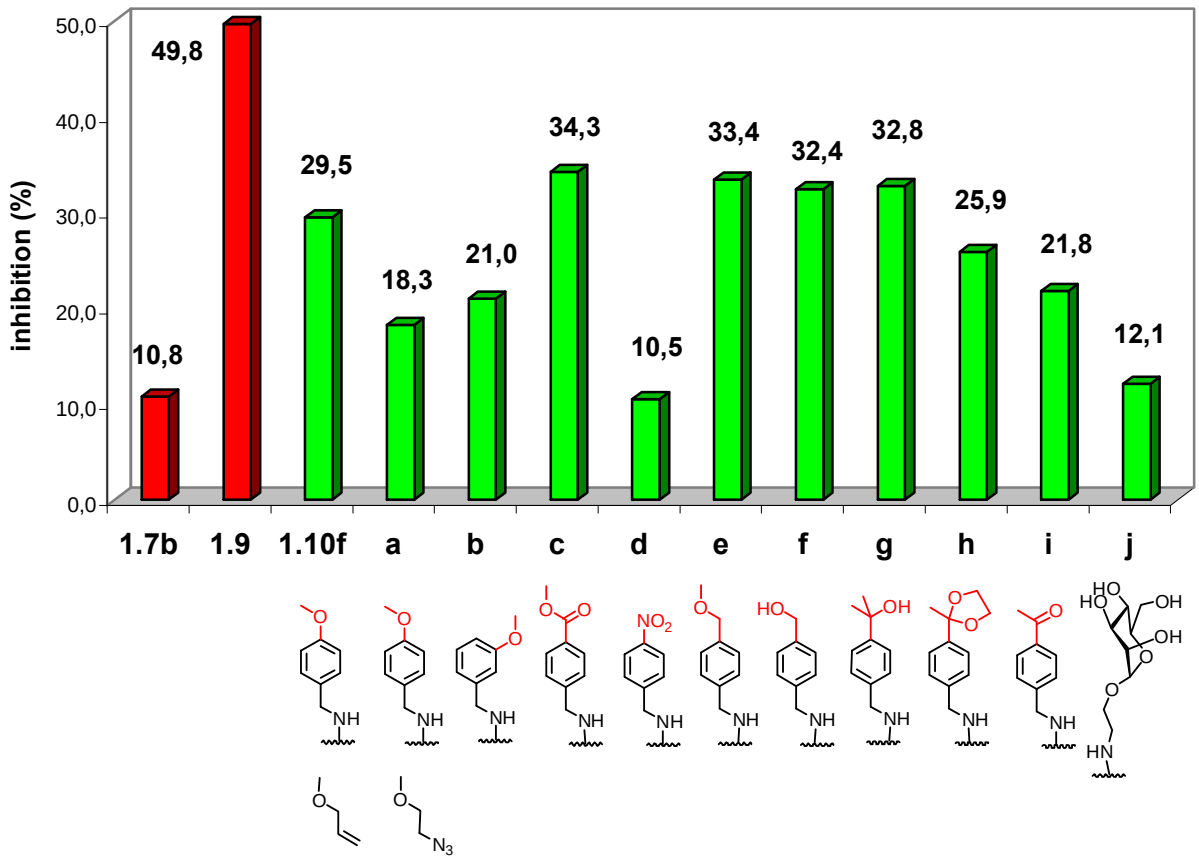
displaying the Manα1-3[Manα1-6]Man trisaccharide. Remaining activated groups were blocked with ethanolamine (1M, 50 μL). The DC-SIGN ECD was expressed and isolated by the Fieschi laboratory[27] using a previously described protocol.[5] The ECD exhibited good affinity (in the μM range) for this surface. This was determined for each chip by a titration curve obtained by flowing increasing protein amount (DC-SIGN ECD) and reporting the response signal as a function of protein concentration

2.1.4.2 Single point SPR experiments

DC-SIGN ligands **2.2a-j** synthetized using the PFP activation sequence (see section 2.1.1) were initially tested by single point SPR experiments in which 150 μM solutions of ligand and a 20 μM solution of DC-SIGN were flown over mannosylated-BSA immobilized on the chip and the competitive inhibition of DC-SIGN was measured. In this experiment the ligands were also compared to the dimethyl ester analogs of the bi and tri-mannoside mimics (**1.7b**, **1.9,** Scheme 2.22), that represent two standards with well-known levels of inhibition.[8,10] These experiments were performed in Grenoble in the group of professor F. Fieschi. The results are shown in graph 2.1 as % of DC-SIGN binding inhibition.



Scheme 2.22 Structures of reference molecules **1.7b** and **1.9** and bisamides **1.10f** and **2.2a-j** measured in single point SPR experiments



Graph 2.1 DC-SIGN Inhibition activities of molecules **1.7b**, **1.9**, **1.10f** and **2.2a-j** (shown as a, b, …) at 150 µM concentration determined by single point SPR measurement with a Man-BSA chip

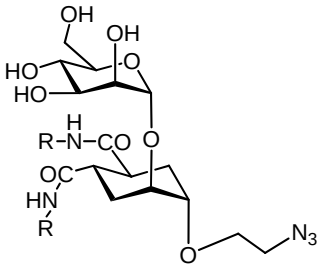
The single point experiments showed that substitution of the benzyl group in para position by -CH_x-O-R groups (**2.2c,e-i**) improves the activity and the best ligands showed inhibition between 32-35 % (c = 150 µM) which is a clear improvement over the activity of the parent dimethyl ester and approaches the activity of the much more synthetically demanding pseudo-trisaccharide **1.9**. On the other hand, the polymanosylated compound **2.2j** had practically the same activity as the reference diester **1.7b** showing that in this case the number of mannose moieties in the molecule has a minor impact on the activity of the ligand. A possible explanation could be that the hydroxyl groups in the mannose residues have to undergo desolvatation before the interaction with the binding site and this process is energetically demanding.

In the single point experiment also the analog of **2.2a**, compound **1.10f,** was tested in which the azidoethanol linker is replaced with an allyl group. This compound had been previously prepared and tested by DC-SIGN adhesion experiment (IC_{50} = 111 µM).[11] In the SPR experiment, derivative **2.2a** showed lower activity than **1.10f** and this confirms that the allyl group has non specific interaction with DC-SIGN. As we have noted above this was also

observed in STD measurements by NMR (Pedro Nieto, Seville). Among the tested ligands compound **2.2f** showed the most promising properties from the viewpoint of activity, solubility and synthesis.

2.1.4.3 IC50 determination by SPR

After the single point SPR experiments showed promising results, the library of bisamides was expanded (see section 2.1.2) and finally, the activities of all bis-amides **2.2a-t** and of the macrocycle **2.47** was measured by determining their IC50 in SPR experiments described above, where the ability of the ligand to inhibit DC-SIGN binding to mannosylated BSA (Man-BSA) was tested. In the assay, Man-BSA is immobilized on a CM4 chip and DC-SIGN is flown in the chamber in the presence of increasing concentrations of the inhibitors thus allowing the determination of the IC50 value. The data were obtained as in several campaigns and compounds **1.7b** and **1.9** were always used as standards. Since these standards exhibited very similar activities in all the SPR experiments (IC50 = app. 0.6 mM for **1.7b** and 0.1 mM for **1.9**) the results can be summarized in table 2.1 and graph 2.2.



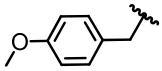
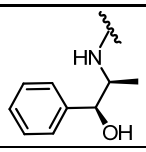
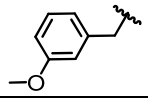
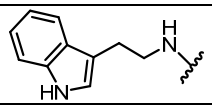
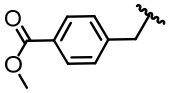
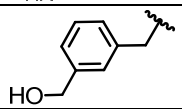
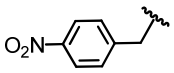
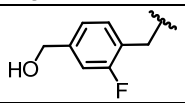
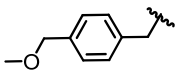
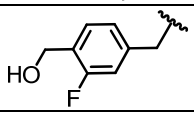
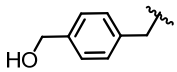
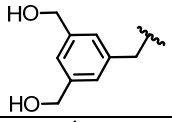
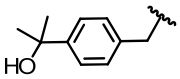
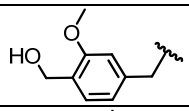
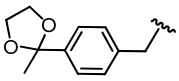
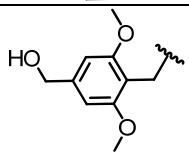
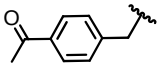
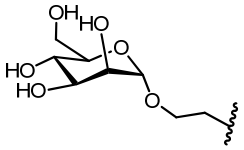
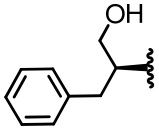
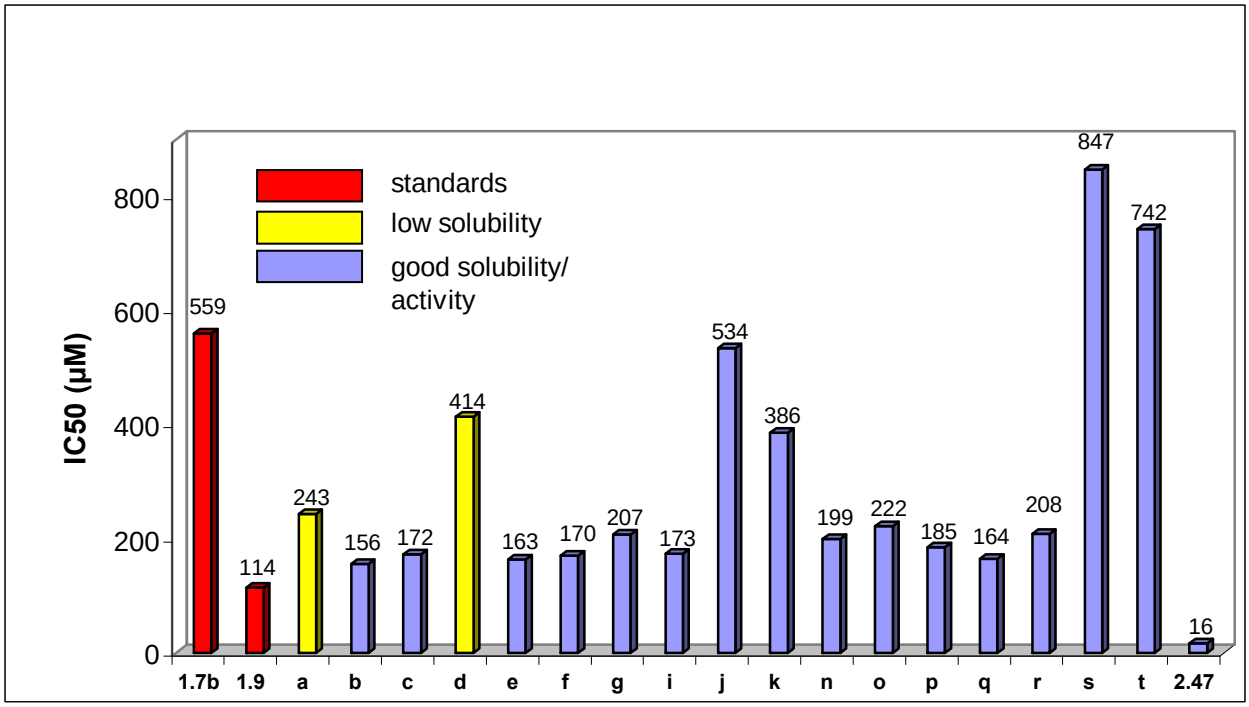
No.	Structure (R=)	IC50 µM[a]	No.	Structure (R=)	IC50 uM[a]
2.2a		243[b]	**2.2l**		nd[d]
2.2b		156	**2.2m**		nd[d]
2.2c		172	**2.2n**		199
2.2d		414[b]	**2.2o**		222
2.2e		163	**2.2p**		185
2.2f		170	**2.2q**		164
2.2g		207	**2.2r**		208
2.2h		nd[c]	**2.2s**		847
2.2i		173	**2.2t**	Diastereoisomer of **2.2f** (Scheme 2.19)	742
2.2j		534	**2.47**	Dimer	16
2.2k		386			

Table 2.1 DC-SIGN ligands **2.2a-t** and **2.47** with IC50 values. a. In SPR competition test with immobilized Man-BSA. b. Low solubility in water. c. Not tested for IC50 due to low stability. d. Insoluble in water



Graph 2.2 IC50 values of final DC-SIGN ligand **2.2a-g,i-k,n-t** (showed as **a**, **b**…) and **2.47** compared to the activity of ligands **1.9** and **1.7b**

Many of the bis-amides prepared showed a remarkable increase in inhibitory activity compared to **1.7b** and some approached the affinity observed for **1.9** a molecule of significantly higher structural and synthetic complexity. Low solubility remains a problem for some of the structures. Molecules **2.2l-m** precipitated in water and their IC50 couldn't be determined. Structures **2.2a** and **2.2d** had some solubility problems too, which was reflected also in their activities. However, the majority of compounds displayed good solubility in water at the concentration required for the assay. The group of bisamides **2.2b-c,e-g,i,n-r** all showed a remarkable activity, as they were found to be only approximately 1.5 fold less potent than **1.9**. As it was already mentioned **2.2f,** featuring an hydroxymethylene group in the *para* position, was selected for structural modifications. The hydroxy group appears to play a role as a H-bond acceptor, since the corresponding methyl ether **2.2e** shows the same inhibition power (cfr **2.2f** and **2.2e**). Addition of fluorine atoms on the ring (**2.2o**, **2.2p**) or of additional lipophylic groups in the proximity of the acceptor (**2.2g**) did not improve significantly the affinity. On the contrary, two methoxy group *meta* to the hydroxymethylene, as in compound **2.2s**, had a marked negative effect, possibly as a result of a different orientation of the aromatic residue around the N-benzylic bond. Similarly, compound **2.2t** with the opposite configuration (1*R*, 2*R, 4R, 5R*)- of the cyclohexane ring interacts poorly with DC-SIGN. Remarkably, dimer **2.47** (1:1 mixture of isomers) with an

IC50 of 16 μM turned out to be the most potent inhibitor of the series, and one of the most effective reported so far. Given that the dimension of the macrocycle in **2.47** is too short to span two consecutive DC-SIGN binding sites (which are separated by ca. 38 Å)[28], the strong activity of this compound is likely derived from a protein aggregation mechanism, which effectively inhibits Man-BSA binding under the assay conditions in this particular model, but may not be relevant in real physiological settings.

Among the tested compound **2.2f** remains the representative of the amide series and the most promising candidate for further elaboration towards multivalent systems. Though the *p*-methylenehydroxy benzyl moiety can show instability in biological systems, further substitution of the benzyl ring (i.e. with fluorine, **2.2o-p**) or substitution of the benzylic position (**2.2g**) could improve its resistance against degradation without significant change in the activity of the ligand.

2.2 Modification of 1.7b at position 6 of the mannose residue

Modification of **1.7b**, by a replacement of its methylester moieties by amides (**2.2a-t**), was proved to be an efficient way to improve the activity and selectivity of the ligand (the selectivity of ligands **2.2a-t** will be discussed in section 2.3). In order to continue on this line and to find further possibilities to enhance the potency of pseudodimannoside based ligands, another modification of **1.7b** was sought. Since the mannose residue of molecule **1.7b** has a close contact with the binding site, a proper modification of its structure could have positive effect on the activity. Based on the available X-ray structure of the DC-SIGN : **1.7b** complex[29] an interesting point to modify would be position 2 of the mannose ring (Figure 2.2).

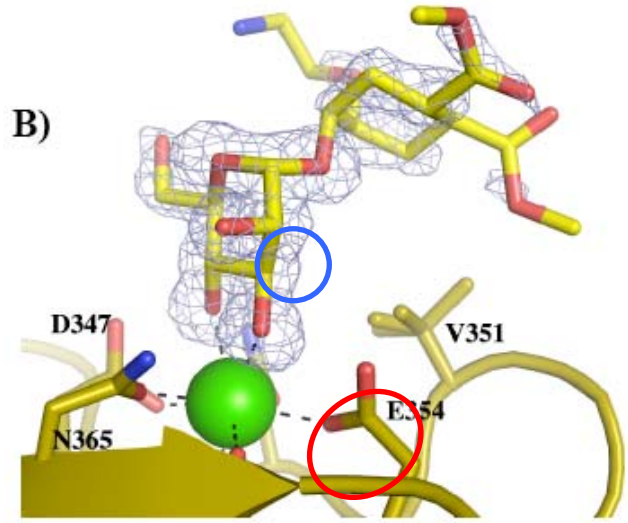
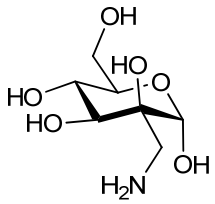


Figure 2.2 X-ray structure of the **1.7b** : DC-SIGN complex. Blue circle: position 2 of the mannose residue. Red circle: carboxylic group of the side chain of glutamic acid E354

The hydroxyl group in position 2 of the mannose ring has axial configuration and therefore a substituent of the hydrogen of the -C<u>H</u>- group at this position would be equatorial allowing it to interact with the side chain of glutamic acid E354. This hypothesis would also explain the remarkable improvement of the activity of compound **1.6c** (k_i = 0.35 mM) in comparison with D-mannose (k_i = 17.1 mM, Scheme 2.23) reported by the Fleet group.[7]



1.6c (K_i = 0.35 mM)

Scheme 2.23 branched D-mannose analogue as potent DC-SIGN inhibitor[7]

However, such a modification would be synthetically very demanding and not very practical. A second point which lends itself to modifications is position 6 in the structure of mannose moiety. A recent publication from Winssinger et. al.[30,31] describes a small library of native mannobioside derivatives, among which a compound with an amino group in position 6 of the non-reducing end was found to be a potent DC-SIGN binder. Docking studies performed within the CARMUSYS network suggests that an amino group at this position could interact efficiently with carboxylic side chains in the vicinity of the Ca^{2+} binding site. Moreover, based on structural studies reported by Feinberg et. al.[32] this position on the non reducing end of Man1-2Man disaccharide **1.8** (see introduction) is relatively close to lysine299 of the binding site of Langherin (Figure 2.3).

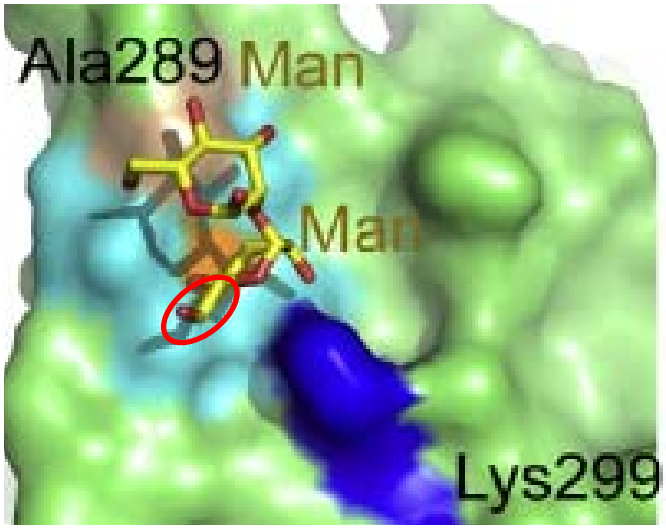
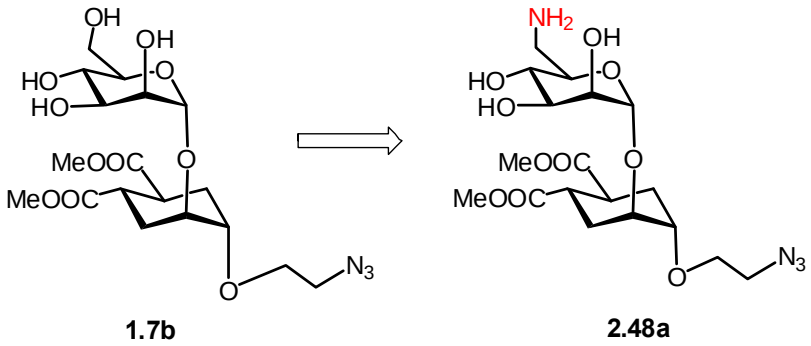


Figure 2.3 Interaction of a Manα1-2Man disaccharide with the binding site of Langherin [32]

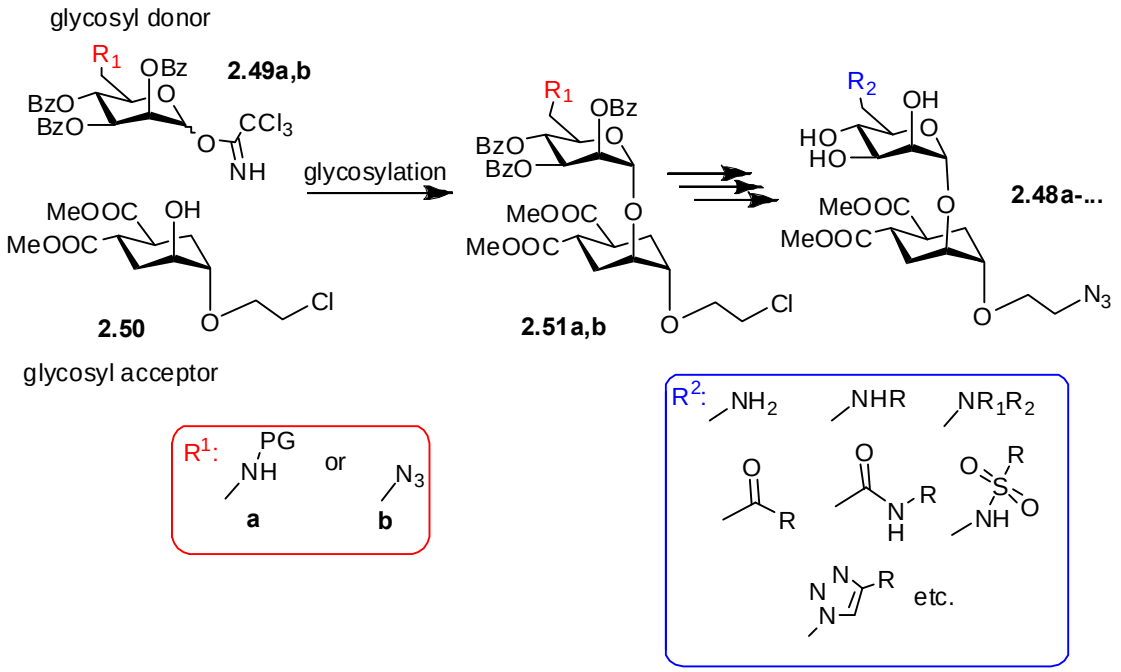
A functional group at position 6 exhibiting repulsive interaction (i. e. a protonated amine group, Scheme 2.24) with lysine299 may result in lower binding activity of the ligand with Langherin and thus improve its selectivity towards DC-SIGN. Furthermore, giving the fact that position 6 is the only primary alcohol in the molecule its modification could be easier in comparison with the secondary alcohols in positions 2-4.



Scheme 2.24 Modification of compound **1.7b** in position 6 of the mannose residue

2.2.1 Synthesis

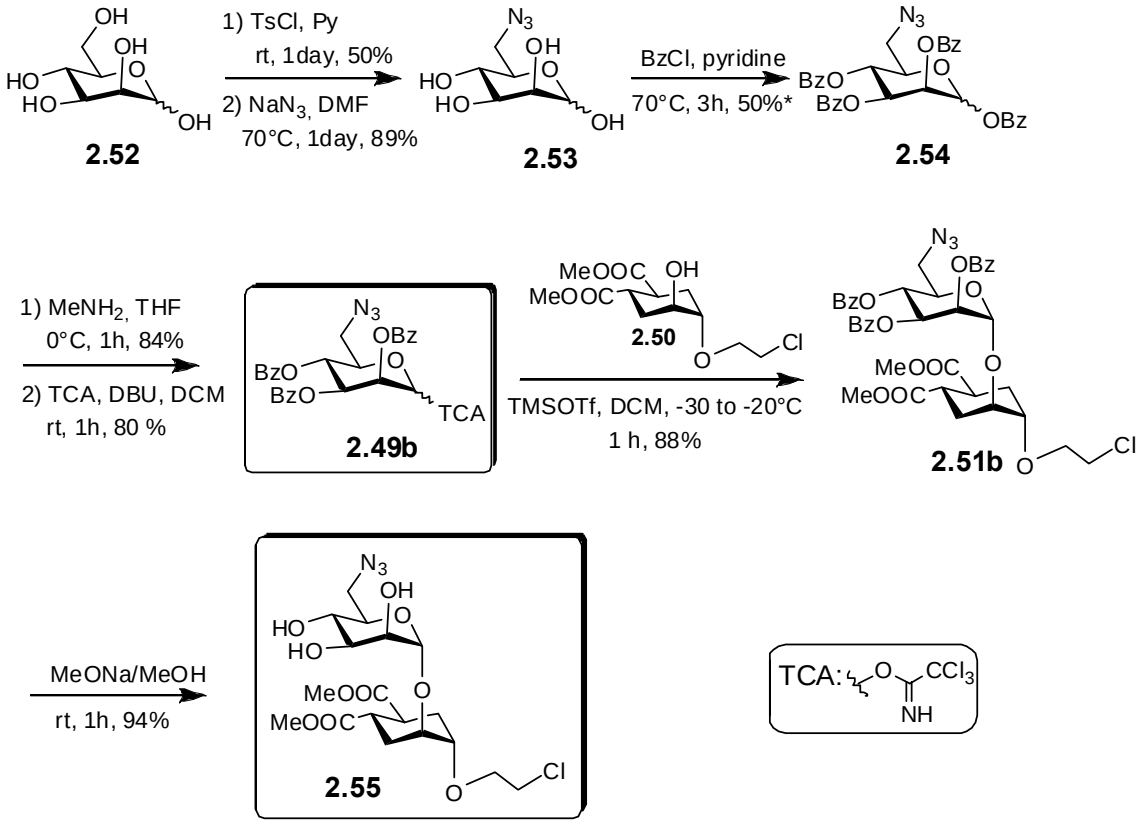
The synthesis of the pseudodimmanoside derivatives **2.2a-t** involves a glycosylation step where the secondary hydroxyl group of the cyclohexyl derivative **2.5** or **2.30** (glycosyl acceptor) is connected with a tetra-O-benzoyl mannose derivative **2.8** (glycosyl donor) activated in anomeric position via a trichloroacetimidate (TCA) (Scheme 2.5 and 2.14). This strategy can also be used to synthesize derivative **2.48**, bearing a protected or masked amine group in position six of the Man residue (Scheme 2.25). After the glycosylation a primary amine could be obtained, which can be further functionalized (secondary and tertiary amines, amides, carbamates, sulfonamides…). For this transformation we envisaged two possible strategies involving either a carbamate or an azide to mask the primary amine function in the manosyl donor **2.49** (Scheme 2.25)**.** So far, only the strategy using an azide (glycosyl donor **2.49b)** as a source of the primary amine has been investigated. To allow orthogonal modification of Man-C6 and of the azidoethanol linker, glycosyl acceptors **2.5** and **2.30** were replaced with the corresponding chloride **2.50** which had been previously obtained.



Scheme 2.25 Synthetic strategy for the preparation of molecules **2.48a,b,…**

The concept of introducing an azide group in position 6 of different aldohexoses has been previously described. The used strategies commonly starts with selective activation of position 6 of an unprotected or partially protected sugar by a tosyl group (Ts) using tosyl chloride in the presence of a base. This reaction has been described among others for β-D-galactose[33] and α-D-glucose[34] both substituted only at the anomeric position (positions 2-5 are unprotected). The synthesis of native mannobiosides modified in position 6 described by Wissinger et. al.[30] also starts with the activation of position 6 of D-mannose (fully unprotected). Since this strategy is used with success in literature, we decided to adopt it with some modification.

The synthesis starts with D-mannose **2.52** (Scheme 2.26) which is selectively activated with a tosyl group in position six using tosyl chloride and pyridine as a base and solvent. This group is substituted by an azide moiety in the following reaction leading to compound **2.53**, which is then fully benzoylated (**2.54**). The anomeric position of **2.54** is deprotected using methylamine giving the α and β mixture of the product. In the subsequent reaction the free hydroxyl group at position 1 is activated using trichloroacetonitrile and DBU as a bulky base. Product **2.49b** represents a glycosyl donor which is treated in the glycosylation step with the glycosyl acceptor **2.50**,[8] which comes from the synthesis of **1.7b** and was available in the laboratory. As a promoter in this step, trimethylsilyl trifluoromethansulfonate (TMSOTf) was added at -30°C and after 1 hour at -20°C the product **2.51b** was isolated in 88% yield. The last step in this reaction sequence is the deprotection of the mannose moiety using sodium methoxide in methanol (Scheme 2.26).

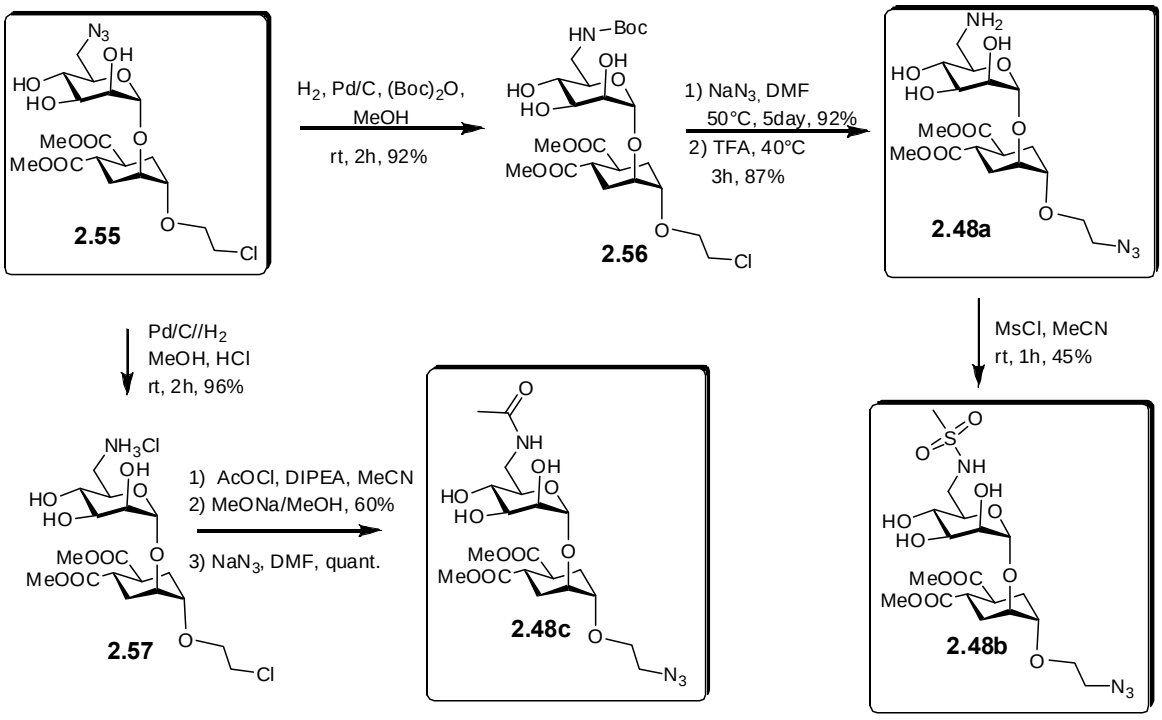


Scheme 2.26 Synthesis of building block **2.55** starting from D-mannose

In order to prepare the desired final ligands, product **2.55** can be modified in several different ways.

In the first route (Scheme 2.27) compound **2.55** is reduced using hydrogen and a catalytic amount of palladium on carbon in the presence of Boc_2O which gives the protected amine in position 6 of mannose, **2.56**. In the following steps the chloride on the ethylene tail is substituted by an azide group and the Boc protecting moiety is removed in acidic condition. Product **2.48a** represents one of the desired final ligands, however, it can be further functionalized, for instance by treatment with mesylchloride which gives derivative **2.48b**, another ligand ready to be tested with DC-SIGN or other lectins .

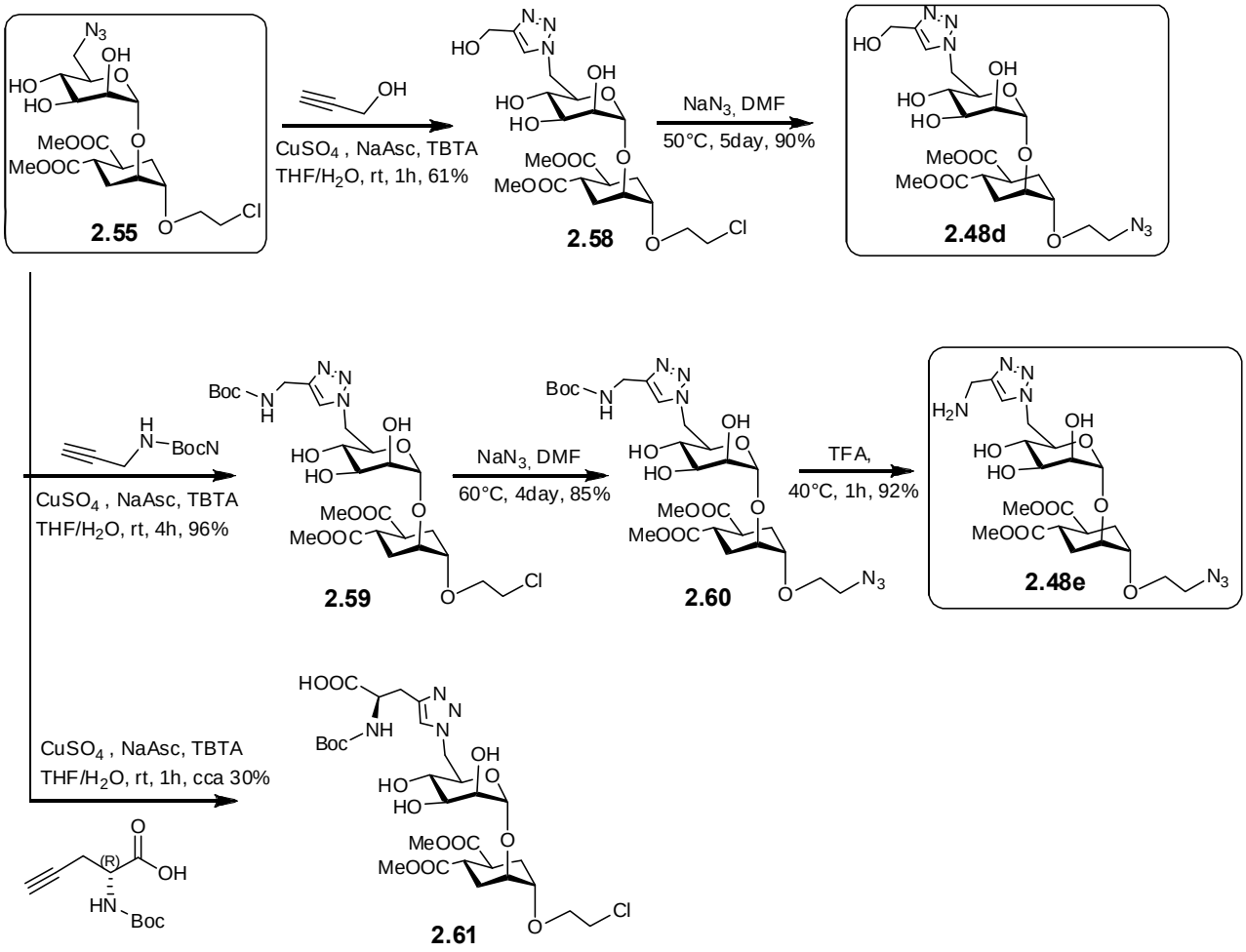
In the second approach (Scheme 2.27) compound **2.55** is reduced with Pd/C without Boc_2O and hence without subsequent protection. The primary amine can be then functionalized with an acetyl group. During this reaction undesired protections of the free hydroxyl groups were observed and therefore the mixture of products was treated with sodium methoxide in order to remove the acetyls from the oxygens. In the last step sodium azide was used to replace the chloride to an azide, resulting in final ligand **2.48c** (Scheme 2.27).



Scheme 2.27 Synthesis of ligands **2.48a-c** starting from building block **2.55**

Compound **2.55** contains an azide function which can be exploited for copper catalysed 1,3 dipolar cycloadition, so called "click" reaction (the concept and principle of the used click reaction will be described more in details in the following chapter).[35,36,37] Propargyl alcohol was the first substrate used for this reaction (Scheme 2.28). As a copper source $CuSO_4$. 5 H_2O was selected, and sodium ascorbate was used to reduce Cu(II) to Cu(I) in situ. Tris-(Benzyltriazolylmethyl)amine (TBTA)[38] is a ligand which efficiently coordinates copper(I) preventing its oxidation and thus enhances the reaction. The final compound **2.48d** was obtained by the treatment of **2.58** with sodium azide which substitutes the Cl function to an azide (Scheme 2.28).

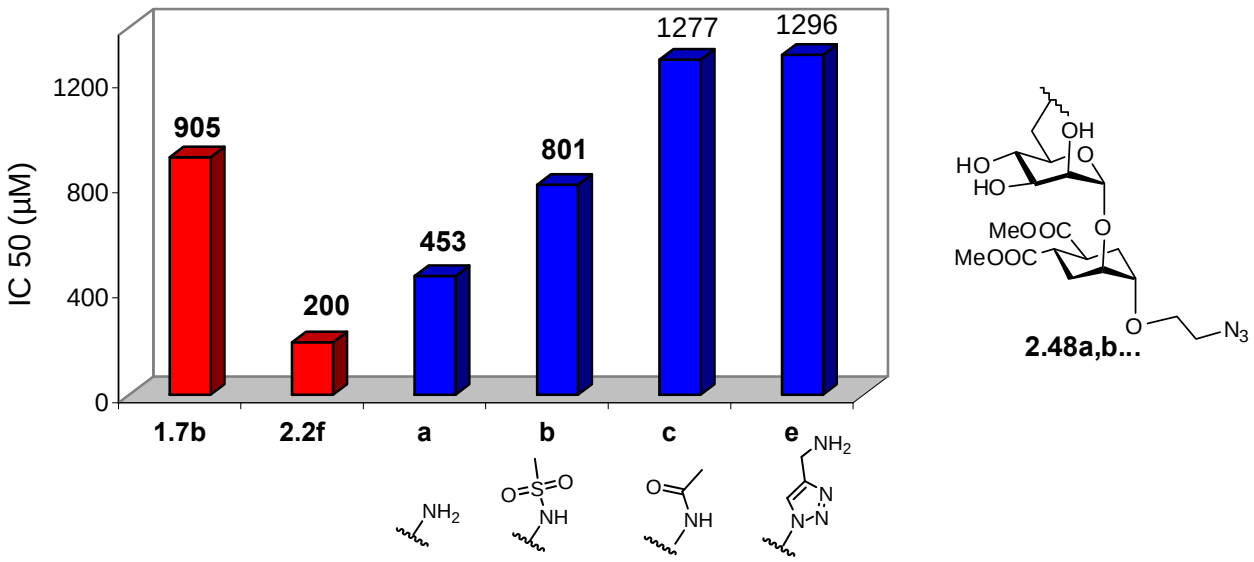
N-Boc protected propargyl amine was also used in the click reaction with **2.55**. The first two steps are similar to those described above (giving products **2.59** and **2.60)** but one additional reaction was done where the primary amine was deprotected using trifluoroacetic acid resulting in final ligand **2.48e**. As a structurally more complex alkyne donor (R)-N-Boc-propargylglycine was used in order to check the scope of the click reaction for our substrate. After one attempt, product **2.61** was obtained in lower yield and due to the little amount of this compound no further reactions were performed (Scheme 2.28).



Scheme 2.28 Synthesis of compounds **2.54d,e** and **2.61** starting from **2.55** using "click" chemistry

2.2.2 Activity determination of DC-SIGN ligands 2.48a-c and d

The prepared ligands **2.48a-c** and **e** where tested by SPR using the same experimental setup described in section 2.1.4. As standards, two molecules were used: the dimannoside mimic **1.7b** and its bisamide derivative **2.2f** Derivatives **2.48b,c** and **e** exhibited slightly lower or very similar activity than the parent pseudo dimannoside **1.7b**. The free primary amine **2.48a** however showed improvement by a factor of two and an IC50 value at 453 μM (Graph 2.3). This observation proves that positive effect on the activity can be achieved by a proper modification of position 6 of the mannose residue, and suggests that a positively charged amino group in this position may have a positive influence also on the activity of **2.2f**.



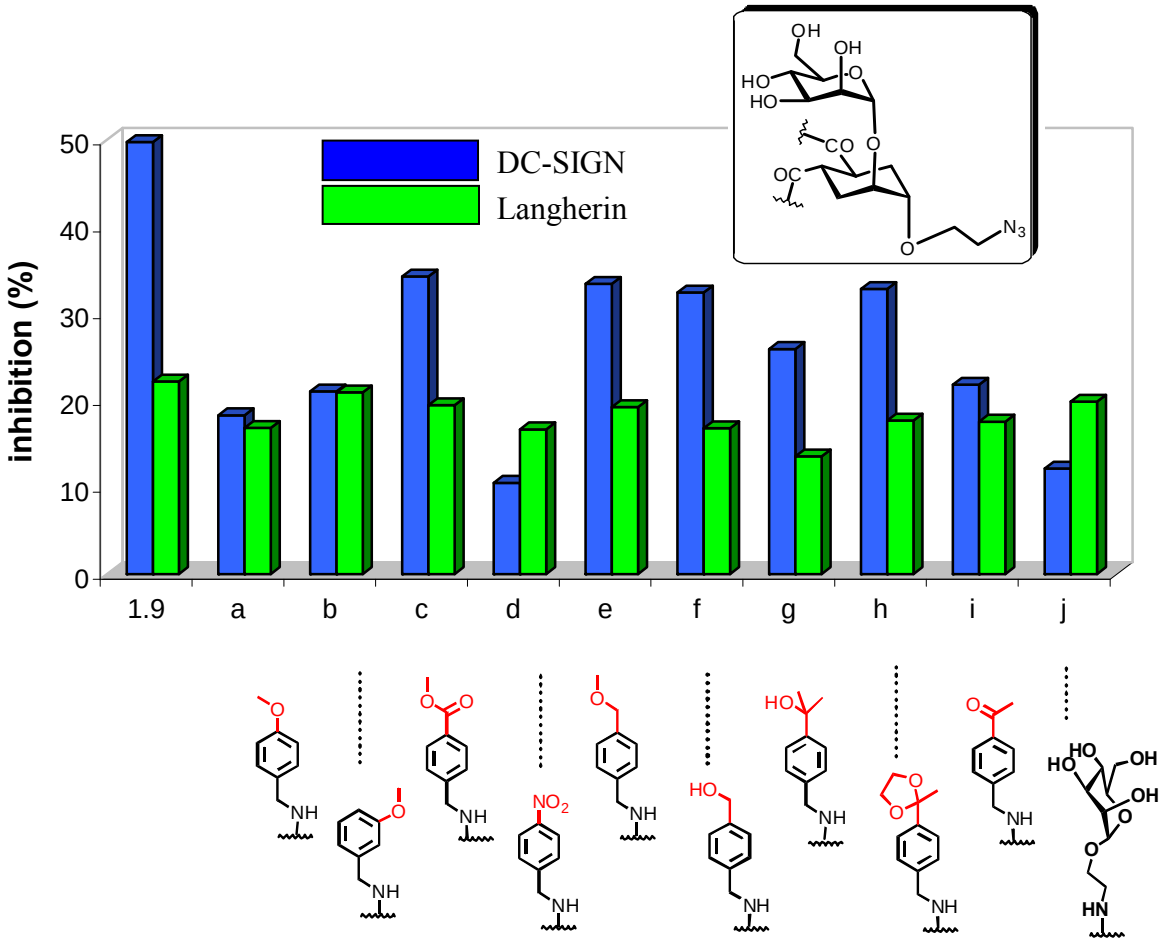
Graph 2.3 IC50 values of ligands **1.7b, 2.2f** and **2.48a-c** and **d** (showed as **a**,**b…**)

In order to achieve further improvements in the activity, the library should be expanded since only a small number of molecules was prepared and tested. However, even these 4 molecules gave us important hints regarding further derivatisation and modification.

2.3 DC-SIGN/Langherin specificity of the pseudodimannoside based ligands

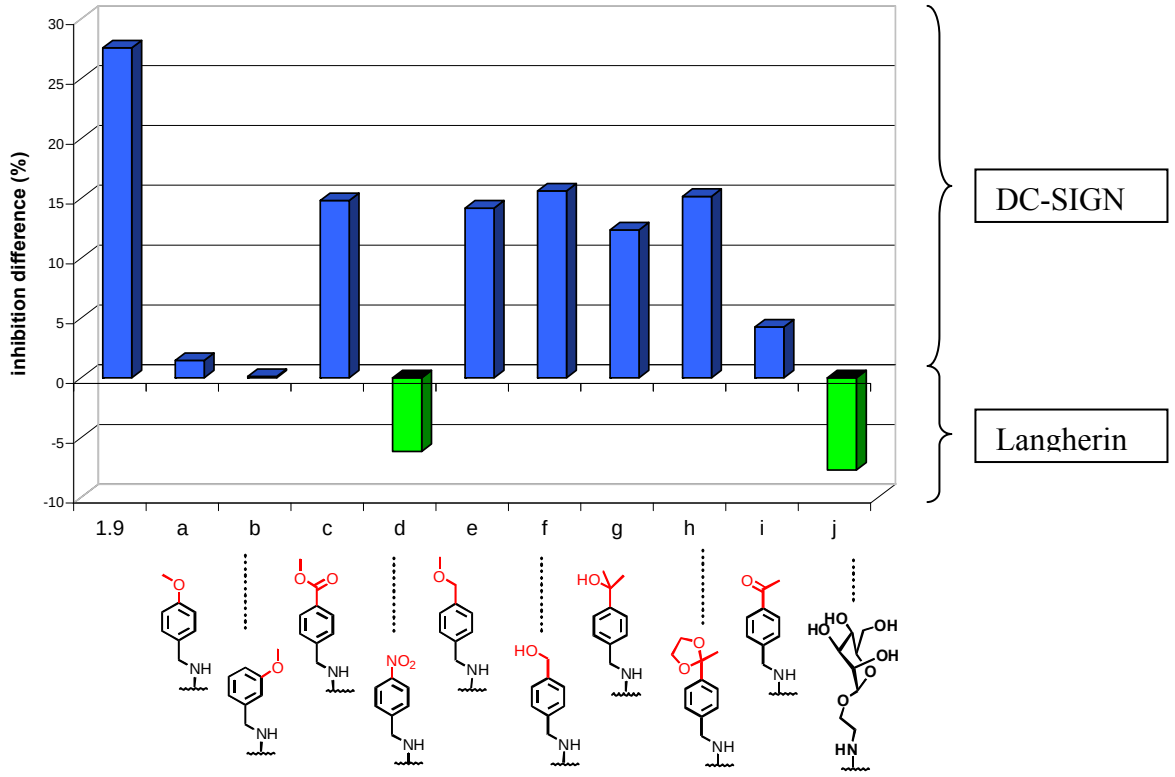
Both DC-SIGN and Langherin are mannose binding lectins involved in the cell mediated immune response. While DC-SIGN acts as a transporter for the HIV virus,[39] Langherin manages to internalize the virus into the Bierbeck granules and destroy it (see introduction).[40] The binding sites of both these receptors contains a Ca^{2+} cation which can coordinate mannose or mannose-containing molecules. However, the different structural environment of each binding site[41,42] should allow to develop ligands which can selectively bind DC-SIGN or Langherin.

In order the investigate the selectivity of the prepared ligands, in the initial experiments single point SPR measurements were performed (group of prof. Frank Fieschi). In this experimental setup 150 µM solutions of ligand and 20 µM solution of lectin were flown over mannosylated-BSA immobilized on the chip and the competitive inhibition of lectins (DC-SIGN or Langherin ECD) was measured. The inhibition activities are summarised in graph 2.4



Graph 2.4 Inhibition levels of DC-SIGN (blue) or Langherin (green) by compounds **1.7b**, **1.9** and **2.2a-j** measured by single point SPR experiment

Substracting Langherin inhibition from DC-SIGN inhibition gives us an indication about the selectivity of the tested ligands.



Graph 2.5 Subtraction of Langherin inhibition from DC-SIGN inhibition of molecules **1.7b**, **1.9** and **2.2a-j** measured by single point SPR experiment

Most of the bis-amides bind better DC-SIGN than Langherin. Compounds **2.2c** and **f** showed the highest selectivity from the amide series in the single point SPR experiment. Practically the same inhibition of DC-SIGN and Langherin was observed in the case of **2.2a** and **b**. On the other hand, the bis p-nitrobenzylamide derivative **2.2d** exhibited better inhibition of Langherin, giving an interesting hint that the selectivity can be tuned by proper substitution of the benzylamide residues. Also, the branched pseudo tetrasaccharide **2.2j** is a better Langherin binder. It was found that the pseudotrisaccharide **1.9** has significant selectivity towards DC-SIGN.

The initial SPR studies revealed, that selectivity towards either tested receptor can be achieved by relatively small modification in the structure. While the mannose moiety is the main farmacophore, its decoration has a significant impact on the binding activity and selectivity of the ligand.

After the single point SPR experiments further studies were carried out (group of Franck Fieschi) and it was observed that the measured DC-SIGN activity of our ligands does not change when SPR chips with different surface density of BSA-Man (competitive inhibitor) is used. However, significant differences were observed with Langherin when the SPR chips were functionalized

with different BSA-Man density. Therefore, it was difficult to determine the absolute IC50 values for Langherin. To overcome this problem an approach was used which quantify the gain of DC-SIGN specificity from one ligand to another. The absolute selectivity of a particular compound ($sel_{\text{comp.}}$) for DC-SIGN vs Langherin can be defined as a ratio of the IC50 values for Langherin and DC-SIGN (Equation 2.1).

$$sel_{comp.} = \frac{IC50_{Langherin}}{IC50_{DC-SIGN}}$$

Equation 2.1

To exclude the effect of the surface density a selectivity gain (*selg*) achieved by switching from one compound to another can be considered:

$$selg_{comp1 \rightarrow comp2} = \frac{sel_{comp2}}{sel_{comp1}}$$

Equation 2.2

This term provides a relative comparison of the improvement of selectivity between the two lectins when switching from one compound to another. Indeed, $selg_{\text{comp.1}\rightarrow\text{comp.2}} > 1$ means a gain of DC-SIGN selectivity *vs*. Langherin for compound 2 compared to compound 1 by a corresponding factor, while values <1 means a loss of selectivity for DC-SIGN. This approach revealed that the selectivity gain is surface density independent.

The first determination of the DC-SIGN/Langherin selectivity gain was performed for the natural mannobioside Manα1-2Man **1.8** and its mimic **1.7b**. It was found that **1.7b** is a more specific DC-SING binder than **1.8** by a factor of 3.

Preliminary studies have been carried out in order to determine the DC-SIGN/Langherin selectivity gain of **2.2f** and **2.48a** over **1.7b**. Considering the obtained IC50 values of the ligands with both the tested lectins, it was calculated that **2.2f** has a selectivity gain over **1.7b** by a factor of 9 and ligand **2.48a** was found to be more specific for DC-SIGN by a factor of 5.8. Further experiments will be performed in order to confirm the obtained data. Nevertheless, the preliminary results are clearly showing high selectivity of compounds **2.2f** and **2.48a** towards DC-SIGN and we can conclude that the novel structures derived from **1.7b** have significantly improved DC-SIGN specificity in comparison with the parent molecule.

2.4 Experimental part

2.4.1 General

Dichloromethane (DCM), methanol (MeOH), N,N-diisopropylethylamine (DIPEA) and triethylamine (TEA) were dried over calcium hydride; THF was distilled over sodium, N,N-dimethylacetamide (DMA) was dried over activated molecular sieves. Reactions requiring anhydrous conditions were performed under nitrogen. ^{1}H and ^{13}C spectra were recorded at 400MHz on a Bruker AVANCE-400 instrument. Chemical shifts (δ) for ^{1}H and ^{13}C spectra are expressed in ppm relative to internal standard ($CDCl_3$: 7.24 for ^{1}H and 77.23 for ^{13}C; CD_3OD: 3.31 for ^{1}H and 49.15 for ^{13}C, D_2O: 4.80 for ^{1}H). Signals were abbreviated as s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet. The numbering used in the NMR characterizations is indicated in the structures showed after the procedures. Sugar signals were numbered as customary; cyclohexane protons are indicated with the letter D followed by numbers. The unusual numbering of the pseudo-disaccharide derivatives in the NMR characterizations was adopted to facilitate comparison with the native disaccharide. In the names of the compounds the conventional numbering is used. Mass spectra were obtained with a ThermoFisherLCQapparatus (ESI ionization), or iontrap ESI Esquire 6000 from Bruker, or a Microflex apparatus (MALDI ionization) from Bruker, or Apex II ICR FTMS (ESI ionization—HR-MS). Specific optical rotation values were measured using a Perkin-Elmer 241, at 589 nm, in a 1 dm cell. Thin layer chromatography (TLC) was carried out with pre-coated Merck F254 silica gel plates. Flash chromatography (FC) was carried out with Macherey-Nagel silica gel 60 (230–400 mesh). For selected compounds the ^{1}H and ^{13}C NMR spectra are showed, the NMR spectra for the rest of the molecules can be found on the compact disc (CD) attached to the thesis.

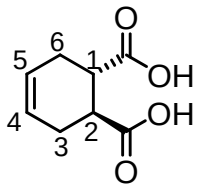
2.4.2 Synthesis of scaffold 2.9 – PFP method

2.4.2.1 Resolution of (1S,2S)-4-Cyclohexene-1,2-dicarboxylic acid, (+)2.1[14, 43]

To a solution of diacid **2.1** (5.12 g, 30.10 mmol, 1 eq, 74% e.e.) in MeOH (35 ml) a solution of quinine (12.40 g, 38.22 mmol, 1.27 eq) in MeOH (13 ml) was added. The resulting solution was stirred at 40°C for 10 minutes then the solvent was removed under reduced pressure. In order to remove the excess of quinine, ethyl acetate was added (25 ml) and the resulting mixture was heated up to reflux for several minutes, then let to cool down to room temperature. The precipitates were filtered to obtain 15.6 g of diacid **2.1**- quinine salt as a white solid.

To the quinine salt methanol (15 ml) was added and heated up to reflux for 20 minutes, then additional 5 ml of methanol was added every 20 minutes until the precipitates doesn't dissolve completely. The mixture was let to cool down to room temperature, then transferred to the freezer to keep at -20°C overnight. The white precipitate was filtered off and washed with the filtrate to obtain 12.5 g of solid which was further recrystallized: methanol (12.5 ml) was added and the resulting mixture was heated to reflux then let to cool to room temperature and kept at -20°C (freezer) for overnight. The recrystallized quinine salt was filtered to obtain 10.3 g of white solid.

In order to liberate the desired diacid **(+)2.1** the quinine salt was dissoved in aqueous HCl solution (10%, 500 ml) and extracted with ethyl acetate (3 x 240 ml). The combined organic phases were washed with aqueous HCl solution (10%, 2 x 50 ml), brine (2 x 40 ml) and water, then dried over sodium sulphate, filtered and concentrated under reduced pressure to obtain 3.08 g of enantiomerically pure product.



(+) 2.1

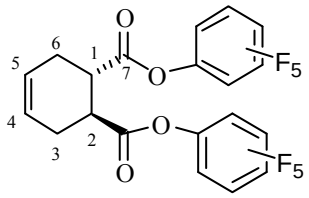
Yield = 82%

$[\alpha]^{20}_{D}$ = + 142 (c = 1.1; EtOH)

^{1}H NMR (400 MHz, $CDCl_3$): 5.73 – 5.66 (m, 2H, H_4, H_5), 2.86 – 2.75 (m, 2H, H_1, H_2), 2.53-2.37 (m, 2H, H_{3eq}, H_{6eq}), 2.29 - 2.10 (m, 2H, H_{3ax}, H_{6ax}).

2.4.2.2 4-cyclohexene-1,2-dicarboxylic acid bispentafluorophenylester, (1*S*,2*S*), 2.3

To a solution of the diacid **2.1**[14] (0.5 g, 2.938 mmol, 1 eq) in dry THF (25 ml) under nitrogen atmosphere EDC•HCl (0.74 g, 3.877 mmol, 3.3 eq.) was added. After 10 minutes pentafluorophenol (1.69 g, 8.815 mmol, 3 eq) was added. The solution was stirred at room temperature for 1 h and then at 40°C for 2 h. After completion of the reaction (TLC, EtOAc) the solvent was removed at reduced pressure and the crude residue was taken up in Et_2O. The organic phase was washed with 1M HCl and saturated Na_2CO_3, then dried over sodium sulphate. The solvent was evaporated at reduced pressure obtaining 1360 mg of pure product as white solid



2.3

Yield = 92%

$[\alpha]^{20}_{D}$ = + 53.6 (c = 0.5; $CHCl_3$)

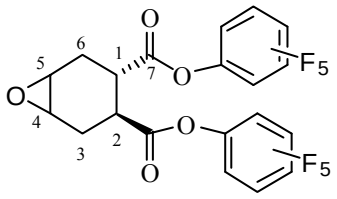
^{1}H NMR (400 MHz, $CDCl_3$): 5.81 (app d, *J*= 2.68 Hz, 2H, H_4, H_5), 3.40-3.31 (m, 2H, H_1, H_2), 2.80-2.68 (m, 2H, $H_{3ps\text{-}eq}$, $H_{6ps\text{-}eq}$), 2.50-2.38 (m, 2H, $H_{3ps\text{-}ax}$, $H_{6ps\text{-}ax}$).

^{13}C NMR (100 MHz, $CDCl_3$): 170.5 (C_7); 142.6 (m, CF); 141.2 (m, CF); 140.1 (m, CF); 139.4 (m, CF); 138.7 (m, CF); 136.9 (m, CF); 124.7 (C_4, C_5); 40.8 (C_1, C_2); 27.8 (C_3, C_6).

^{19}F-NMR (282 MHz, $CDCl_3$): -153.2 (d, 2F, F_{ortho}, $J_{o\text{-}m}$= 20 Hz), -157.9 (t, 1F, F_{para}, $J_{p\text{-}m}$= 22.5 Hz), -162.4 (t, 2F, F_{meta}).

2.4.2.3 7-Oxabicyclo[4.1.0]heptane-3,4-dicarboxylic acid, bispentafluorophenyl ester (3*S*,4*S*), 2.4

To a solution of the PFP ester **2.3** (1360 mg, 2.7 mmol, 1 eq.) in dry DCM (6 ml), 77% MCPBA was added (788 mg, 3.5 mmol, 1.3 eq). The reaction was stirred under nitrogen atmosphere at room temperature. After completion of the reaction (16 h, TLC 8:2 Hex:EtOAc), the solvent was removed at reduced pressure, the reaction mixture was diluted with Et_2O and washed with sat. $NaHCO_3$ and water. The organic phase was dried over sodium sulphate and the solvent was removed at reduced pressure. The crude product was purified by flash chromatography (8:2 Hex:EtOAc) leading to 1162 mg of pure product.



2.4

Yield: 83%

$[\alpha]^{20}_{D}$ = + 37.7 (c = 0.5; $CHCl_3$)

MS (FAB) calculated for $[C_{20}H_9F_{10}O_5]^+$: 519; found = 519.

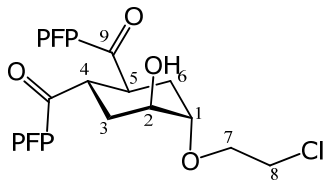
^{1}H NMR (400 MHz, $CDCl_3$): 3.43-3.34 (m, 2H, H_4 or H_5, H_2 or H_1), 3.32 (dd, 1H, H_5 or H_4, $J_{5\text{-}4}$ = $J_{5\text{-}6}$ or $J_{4\text{-}3}$ = 4 Hz), 3.14 (dt, 1H, H_2 or H_1, $J_{2\text{-}3eq}$ or $J_{1\text{-}6eq}$ = 6.8 Hz, $J_{2\text{-}3ax}$ or $J_{1\text{-}6ax}$ = $J_{2\text{-}1}$ = 10.0 Hz), 2.76 (ddd, 1H, H_{3eq} o H_{6eq}, $J_{3eq\text{-}4}$ or $J_{6eq\text{-}5}$ = 1.6 Hz, $J_{3eq\text{-}2}$ or $J_{6eq\text{-}1}$ = 5.2 Hz, J_{gem} = 14.8 Hz), 2.61 (ddd, 1H, H_{6eq} or H_{3eq}, $J_{6eq\text{-}1}$ or $J_{3eq\text{-}2}$ = 4.4 Hz, J_{gem} = 15.6 Hz), 2.37 (dd, 1H, H_{6ax} or H_{3ax}), 2.16 (ddd, 1H, H_{3ax} or H_{6ax}, $J_{3ax\text{-}4}$ or $J_{6ax\text{-}5}$ = 2.0 Hz).

^{13}C NMR (100 MHz, $CDCl_3$): 170.6, 169.4 (C_7); 142.6 (m, CF); 141.1 (m, CF); 140.0 (m, CF); 139.4 (m, CF); 138.7 (m, CF); 137.3 (m, CF); 51.5, 50.1 (C_4, C_5); 39.4, 37.5 (C_1, C_2); 26.9, 26.2 (C_3, C_6).

^{19}F-NMR (282 MHz, $CDCl_3$): -153.1 (dd, 2F, F_{ortho}, $J_{o\text{-}m}$= 84.6 Hz, $J_{o\text{-}p}$= 16.9 Hz), -157.6 (m, 1F, F_{para}), -162.3 (m, 2F, F_{meta}).

2.4.2.4 1,2-Cyclohexanedicarboxylic acid, 5-(2-chloroethoxy)-4-hydroxy, 1,2-bispentafluorophenyl ester, (1*S*,2*S*,4*S*,5*S*), 2.5

To a solution of the epoxide **2.4** (1162 mg, 2.24 mmol, 1 eq) in minimum amount of dry DCM (1 ml) under nitrogen atmosphere 2-chloroethanol (0.75 mL, 115.1 mmol, 50 eq.) and $Cu(OTf)_2$ (162 mg, 0.448 mmol, 0.2 eq.) were added. The solution was stirred at room temperature. After completion of the reaction in (16 h, TLC 7:3 Hex:EtOAc) the solvent was removed under reduced pressure and the crude residue was purified by flash chromatography (8:2 Hex:EtOAc) obtaining 1266 mg of pure product as colorless wax which later solidified.



2.5

Yield: 94%

$[\alpha]^{20}_D$ = +3.93 (c = 0.57; $CHCl_3$)

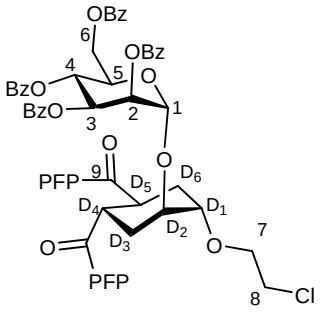
MS (FAB) calculated for $[C_{22}H_{13}ClF_{10}O_6Na]^+$: 621; found: 621.

^{1}H NMR (400 MHz, $CDCl_3$): 4.13 (m, 1H, H_2), 3.92 (m, 1H, H_{7b}), 3.77 (m, 1H, H_{7a}), 3.66 (m, 2H, H8), 3.63 (m, 1H, H_1) 3.62 - 3.50 (m, 2H, H_4, H_5), 2.34 - 2.27 (m, 2H, H_6), 2.27 - 2.22 (m, 2H, H_3), 1.82 (br s, 1H, OH).

^{13}C NMR (100 MHz, $CDCl_3$): 170.4, 170.4 (C_9); 142.4 (m, CF); 141.1 (m, CF); 139.9 (m, CF); 139.3 (m, CF); 138.6 (m, CF); 136.8 (m, CF); 76.6 (C_1); 69.8 (C_7); 66.58 (C_2); 43.3 (C_8); 39.1 (C_4); 38.6 (C_5); 30.6 (C_3); 27.4 (C_6).

2.4.2.5 1,2-Cyclohexanedicarboxylic acid, 4-(2-chloroethoxy)-5-[(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)oxy]-, 1,2- bispentafluorophenyl ester, (1*S*,2*S*,4*S*,5*S*), 2.9

A mixture of the acceptor **2.5** (300 mg, 0.5 mmol, 1 eq.) and the donor **2.8** (445 mg, 0.6 mmol, 1.2 eq.) was co-evaporated with toluene three times, then acid washed and powdered molecular sieves 4Å were added; the mixture was kept under vacuum for few h and then dissolved in dry CH_2Cl_2 (4 ml). After cooling at -20°C, TMSOTf (18 μL, 0.1 mmol, 0.2 eq.) was slowely added and the reaction mixture was stirred at that temperature. The reaction completition (1 h, TLC Hex:EtOAc = 7:3) the reaction was quenched with NEt_3. The mixture was warmed to room temperature, filtered through a celite pad and concentrated at reduced pressure. The crude product was purified by flash chromatography (silica, hexane with gradient of ethyl acetate from 10 to 20 %) affording 407 mg of pure product.



2.9

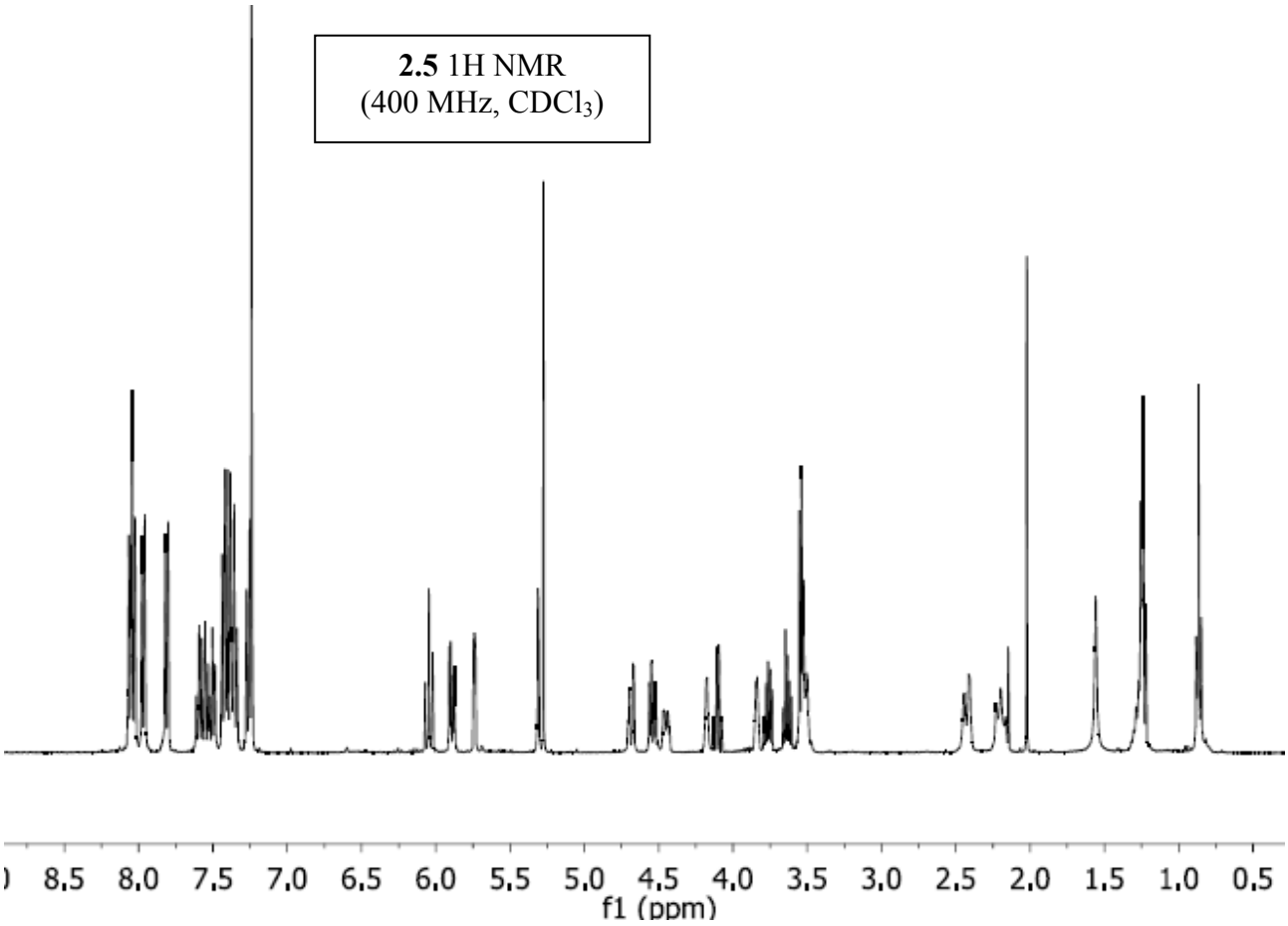
Yield: 67 %.

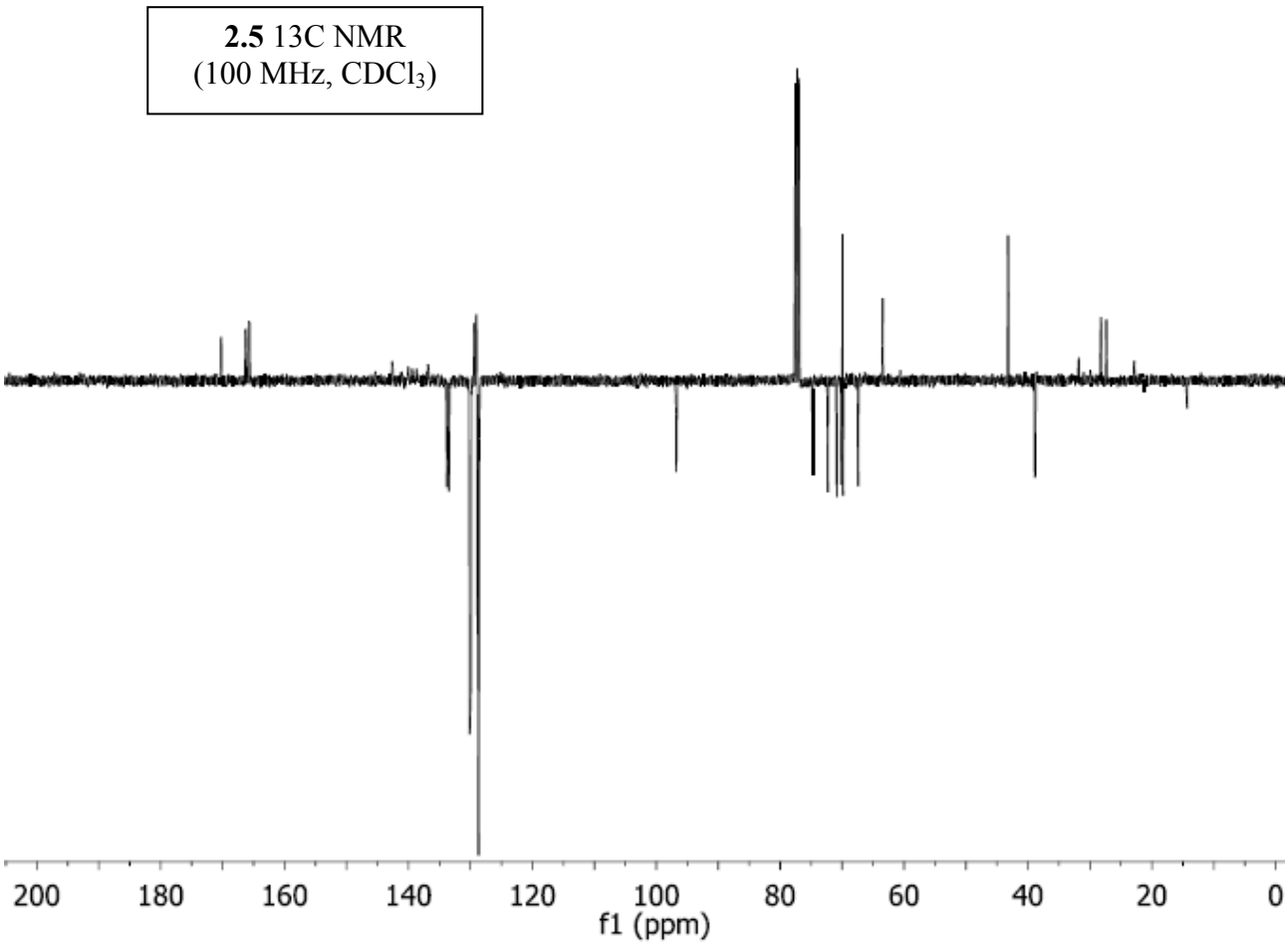
$[\alpha]^{20}_D$ = -26.7 (c = 0.55; $CHCl_3$)

MS (FAB): calculated for$[C_{56}H_{39}ClF_{10}O_{15}Na]^+$: 1199; found: 1199

^{1}H NMR (400 MHz, $CDCl_3$): 8.08 (m, 4H, H_{Bz}), 8.99 (d, 2H, H_{Bz}, J = 7.2 Hz), 7.84 (d, 2H, H_{Bz}, J = 7.2 Hz), 7.66 - 7.50 (m, 3H, H_{Bz}), 7.49 - 7.34 (m, 6H, H_{Bz}), 7.34 - 7.23 (m, 3H, H_{Bz}), 6.08 (t, 1H, H_4, $J_{4\text{-}3} = J_{4\text{-}5}$= 10.0 Hz), 5.92 (dd, 1H, H_3, $J_{3\text{-}4}$ = 10 Hz, $J_{3\text{-}2}$= 3.2 Hz), 5.77 (dd 1H, H_2, $J_{2\text{-}3}$ = 3.2 Hz, $J_{2\text{-}1}$= 1.2 Hz, 5.34 (d, 1H, H_1, $J_{1\text{-}2}$= 1.2 Hz), 4.72 (dd, 1H, H_{6b}, $J_{6\text{-}5}$ = 2.8 Hz, $J_{6a\text{-}6b}$ = 12.0 Hz), 4.57 (dd, 1H, H_{6a}, $J_{6\text{-}5}$ = 5.2 Hz, $J_{6a\text{-}6b}$ = 12.0 Hz), 4.49 (m, 1H, H_5), 4.21 (m, 1H, D_2), 3.87 (m, 1H, D_1), 3.79 (m, 1H, H_{7a}), 3.66 (m, 1H, H_{7b}), 3.59 - 3.50 (m, 4H, H_{8a}, H_{8b}, D_4, D_5), 2.50 - 2.40 (m, 2H, D_{3eq}, D_{6eq}), 2.28 - 2.17 (m, 2H, D_{3ax}, D_{6ax}).

^{13}C NMR (100 MHz, $CDCl_3$): 170.1, 170.1 (C_9); 166.2, 165.7, 165.6, 165,5 (CO_{BZ}); 142.5 (m, CF); 141.1 (m, CF); 139.9 (m, CF); 139.2 (m, CF); 138.6 (m, CF); 136.8 (m, CF); 133.8, 133.7, 133.5, 133.4 (CH_{BZ}); 130,0 130.0, 129.9, 129.9 (CH_{BZ}); 129.3, 129.1, 129.0, 129.0 ($Cquat_{BZ}$); 128.8, 128.6, 128.6, 128.5 (CH_{BZ}); 96.7 (C_1); 74.7 (C_{D1}); 72.3 (C_{D2}); 70.8 (C_2); 70.0 (C_3); 69.9 (C_7); 69.8 (C_5); 67.4 (C_4); 63.4 (C_6); 43.1 (C_8); 38.8, 38.7 (C_{D4}, C_{D5}); 28.2 (C_{D3}); 27.3 (C_{D6}).

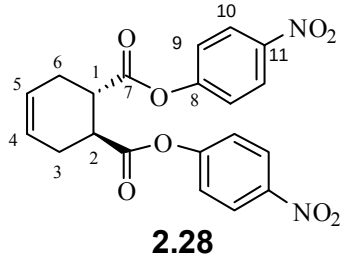




2.4.3 Synthesis of scaffold 2.31 – PNP methodology

2.4.3.1 4-cyclohexene-1,2-dicarboxylic acid bis(4-nitro)phenylester, (1*S*,2*S*), 2.28

EDC·HCl (394 mg, 2.05 mmol, 3.5 eq.) was added to a solution of diacid **2.1**[14] (100 mg, 0.59 mmol, 1 eq.) in dry THF (5.8 ml) under stirring and under a nitrogen atmosphere. After 10 minutes p-nitrophenol (245 mg, 1.76 mmol, 3 eq.) was added. The solution was stirred at room temperature for 2 h. After completion of the reaction the solvent was evaporated under reduced pressure, the residue was taken up in Et_2O, washed with 1M HCl, saturated Na_2CO_3 (3x) and water; then dried over sodium sulphate. Solvent was evaporated under reduced pressure to yield pure product **2.28** as a pale yellow solid.



2.28

Yield: 70%

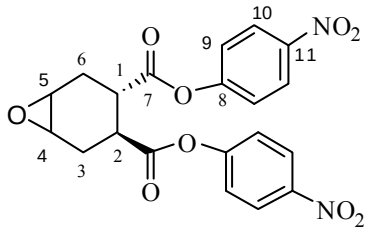
$[\alpha]_D^{20}$ = + 129.6 (c = 1 in chloroform).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.28 - 8.22 (m, 4H, H_{10}), 7.27 - 7.22 (m, 4H, H_9), 5.83 (app d, *J* = 2.8 Hz, 2H, H_4, H_5), 3.27 - 3.19 (m, 2H, H_1, H_2), 2.78 - 2.68 (m, 2H, $H_{3ps\text{-}eq}$, $H_{6ps\text{-}eq}$), 2.48 - 2.37 (m, 2H, $H_{3ps\text{-}ax}$, $H_{6ps\text{-}ax}$).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.8 (C_7); 155.4 (C_{11}); 145.7 (C_8); 125.5 (C_{10}); 124.9 (C_5, C_4); 122.5 (C_9); 41.5 (C_1, C_2); 28.0 (C_3, C_6).

2.4.3.2 7-Oxabicyclo[4.1.0]heptane-3,4-dicarboxylic acid bis(4-nitro)phenylester (3*S*,4*S*), 2.29

MCPBA (77%, 891 mg, 3.98 mmol, 1.2 eq.) was added to a solution of the PNP ester **2.28** (1367 mg, 3.32 mmol, 1 eq.) in dry CH_2Cl_2 (11 ml) under stirring. The reaction was stirred under nitrogen at room temperature for 16 h. The solvent was evaporated at reduced pressure, the reaction mixture was diluted with EtOAc and washed with saturated $NaHCO_3$ (3x) and with water. The organic phase was dried over sodium sulphate and the solvent evaporated under reduced pressure to yield of pure product **6** as white solid.



2.29

Yield: 96%

$[\alpha]_D^{20}$ = + 82.2 (c = 1.1 in chloroform).

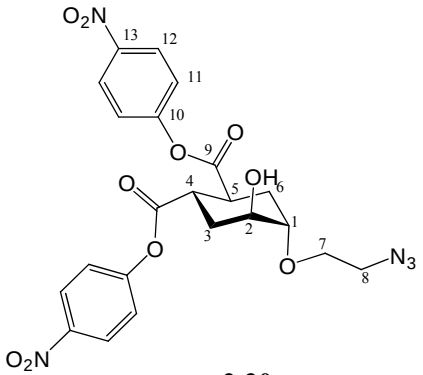
MS (ESI) calculated for $[C_{20}H_{16}N_2O_9Na]^+$: 451.3; found: 452.0

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.28 - 8.22 (m, 4H, H_{10}), 7.27 - 7.21 (m, 4H, H_9), 3.43 – 3.36 (m, 1H, H_4 or H_5), 3.35 - 3.31 (m, 1H, H_4 or H_5), 3.32 - 3.24 (m, 1H, H_1 or H_2), 3.08 - 3.00 (m, 1H, H_1 or H_2), 2.79 – 2.71 (m, 1H, H_{3eq} or H_{6eq}), 2.63 – 2.54 (m, 1H, H_{3eq} or H_{6eq}), 2.42 – 2.33 (m, 1H, H_{3az} or H_{6az}), 2.21 – 2.12 (m, 1H, H_{3ax} or H_{6az}).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.6, 171.5 (C_7); 155.4, 155.3 (C_8); 149.9 (C_{11}); 125.5 (C_{10}); 122.5, 122,6 (C_9); 51.8, 50.3 (C_4, C_5); 40.1, 38.1 (C_1, C_2); 26.8, 26.3 (C_3, C_6).

2.4.3.3 1,2-Cyclohexanedicarboxylic acid, 4-hydroxy-5-(2-azidoethoxy)-, 1,2-bis(4-nitro)phenyl ester, (1*S*,2*S*,4*S*,5*S*), 2.30

To the solution of 2-azidoethanol **2.33**[20] (cca 600 mg, 7 mmol, 58 eq) in DCM (3 ml) epoxide **2.29** (50 mg, 0.12 mmol, 1 eq) and $Cu(OTf)_2$, (4 mg, 0.01 mmol, 0.1 eq.) were added and stirred under nitrogen at room temperature. After completion (16 h) the solvents were removed under reduced pressure and the crude was purified by flash chromatography (hexane with gradient of ethyl acetate from 20 % to 50 %) to yield pure product **2.30** as colourless wax.



2.30

Yield: 70%

$[\alpha]_D^{20}$ = + 33.8 (c = 1.1 in chloroform)

MS (ESI) calculated for $[C_{22}H_{21}N_3O_{10}Na]^+ = [(M - N_2) + Na]^+$: 512.4; found: 512.0.

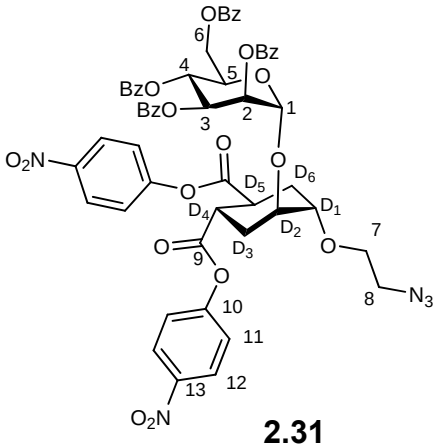
^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.27 - 8.17 (m, 4H, H_{12}), 7.31 - 7.23 (m, 4H, H_{11}), 4.19 - 4.14 (m, 1H, H_2), 3.88 – 3.82 (m, 1H, H_{7a}), 3.73 – 3.63 (m, 2H, H_{7b}, H_1), 3.49 – 3.29 (m, 4H, H_4, H_5, $H_{8a,b}$), 2.40 – 2.10 (m, 4H, H_3, H_6).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.7, 172.6 (C_9); 155.5 (C_{10}); 145.8, 145.7 (C_{13}); 125.5 (C_{12}); 122.6, 122,6 (C_{11}); 76.5 (C_1); 68.7 (C_7); 66.3 (C_2); 51.1 (C_8); 39.4, 39.1 (C_4, C_5); 30.5, 27.0 (C_3, C_6).

2.4.3.4 1,2-Cyclohexanedicarboxylic acid, 4-(2-azidoethoxy)-5-[(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)oxy]-, 1,2- bis(4-nitro)phenyl ester, (1*S*,2*S*,4*S*,5*S*), 2.31

A mixture of the acceptor **2.30** (37 mg, 0.071 mmol, 1 eq.) and the donor **2.8**[16] (65 mg, 0.086 mmol, 1.2 eq.) was coevaporated with toluene three times. Powdered and activated acid washed 4Å molecular sieves were added; the mixture was kept under vacuum for a few h and then dissolved with dry CH_2Cl_2 (1 mL). After cooling at −20°C, TMSOTf (3μL, 0.014 mmol, 0.2 eq.)

was added to the reaction mixture under stirring. The reaction was stirred at -20°C for 1 h. The reaction was quenched with Et_3N and the mixture warmed to room temperature and filtered over a celite pad. The filtrate was evaporated at reduced pressure and the crude product purified by flash chromatography (toluene with gradient of ethyl acetate from 0 % to 10 %) to yield pure product **2.31** as white foam.



2.31

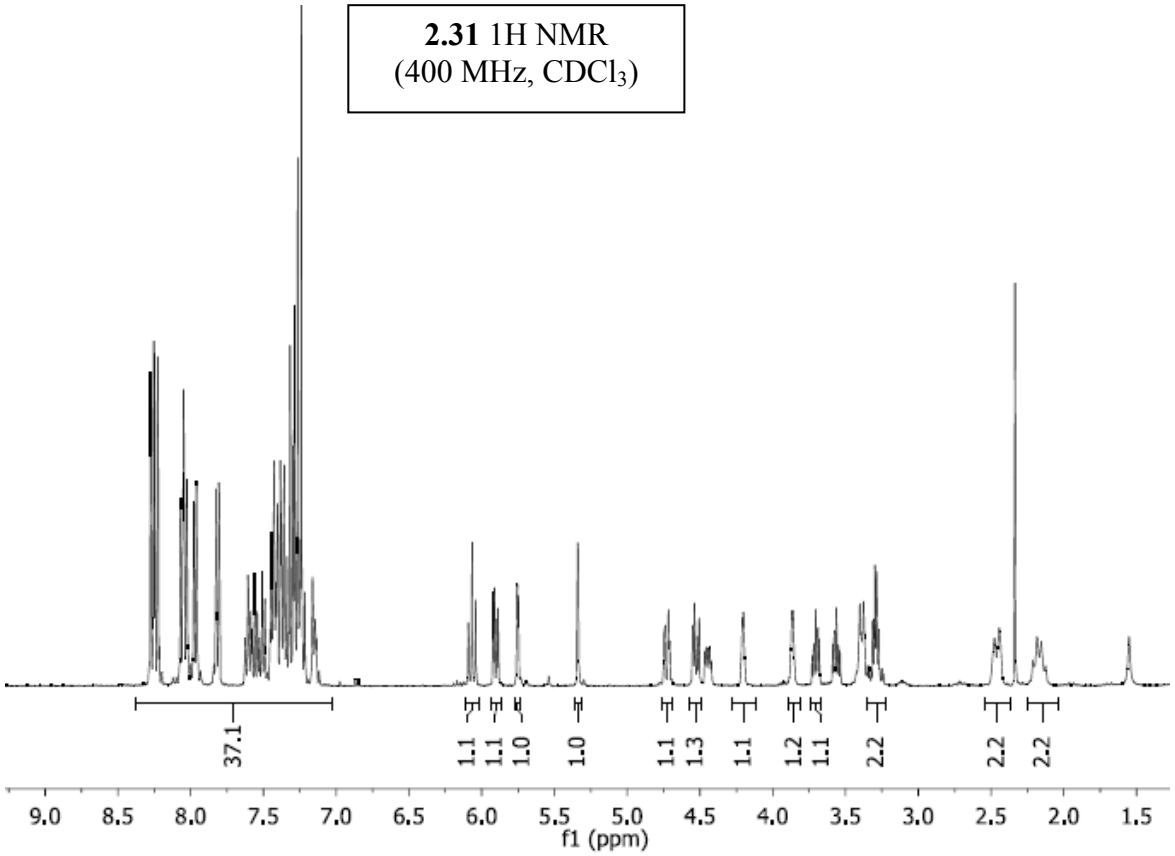
Yield: 85 %

$[\alpha]_D^{20}$ = - 18.0 (c = 0.5 in chloroform).

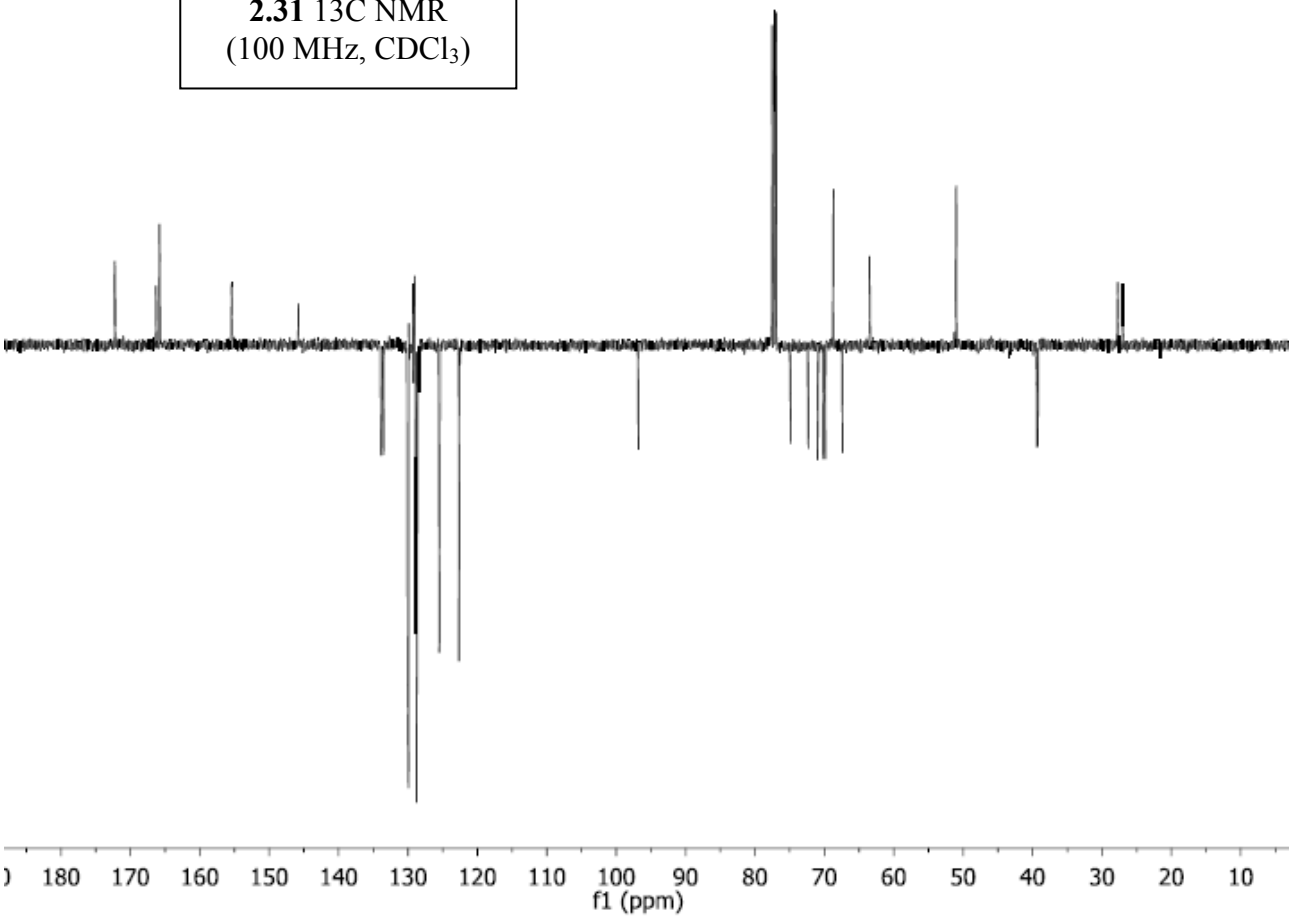
MS (ESI) calculated for $[C_{56}H_{47}N_5O_{19}Na]^+$: 1117.0; found 1116.1.

^{1}HNMR (400 MHz, $CDCl_3$): δ = 8.30 – 8.20 (m, 4H, H_{12}), 8.09 – 8.00 (m, 4H, H_{Bz}), 7.99 – 7.94 (m, 2H, $H_{Bz,}$), 7.83 – 7.79 (m, 3H, H_{Bz}), 7.63 – 7.48 (m, 3H, H_{Bz}), 7.46 – 7.20 (m, 12H, H_{Bz}, H_{11}), 6.07 (t, 1H, H_4, $J_{4\text{-}3} = J_{4\text{-}5}$= 10.0 Hz), 5.90 (dd, 1H, H_3, $J_{3\text{-}4}$ = 10.0 Hz, $J_{3\text{-}2}$ = 3.3), 5.75 (dd, 1H, H_2, $J_{2\text{-}1}$ = 1.7 Hz, $J_{2\text{-}3}$ = 3.3 Hz), 5.34 (d, 1H, H_1, $J_{1\text{-}2}$ = 1.7 Hz), 4.73 (dd, 1H, H_{6b}, $J_{6b\text{-}5}$ = 2.9 Hz, $J_{6a\text{-}6b}$ = 12.0 Hz), 4.53 (dd, 1H, H_{6a}, $J_{6a\text{-}5}$ = 5.3 Hz, $J_{6a\text{-}6b}$ = 12 Hz), 4.48 - 4.40 (m, 1H, H_5), 4.23 - 4.18 (m, 1H, D_2), 3.89 – 3.84 (m, 1H, D_1), 3.74 – 3.67 (m, 1H, H_{7a}), 3.60 – 3.52 (m, 1H, H_{7b}), 3.44 - 3.34 (m, 2H, D_4, D_5), 3.35 - 3.23 (m, 2H, H_8), 2.52 - 2.41 (m, 2H, D_{3eq}, D_{6eq}), 2.25 - 2.09 (m, 2H, D_{3ax}, D_{6ax}). **^{13}C NMR** (100 MHz, $CDCl_3$): δ = 172.3, 172.2 (C_9); 166.3, 165.8, 165.7 (CO_{BZ}); 155.5, 155.4 (C_{10}); 145.8 (C_{13}); 133.9, 133.8, 133.6, 133.5 (CH_{BZ}); 130.1, 130.1, 129.9, 129.9, 129.9 (CH_{BZ}); 129.3, 129.2, 129.1, 129.0 ($Cquat_{BZ}$); 128.9, 128.7, 128.9, 128.4 (CH_{BZ}); 125.5, 125.4 (C_{12}); 122.7, 122.6 (C_{11}); 96.8 (C_1); 74.9 (C_{D1}); 72.3 (C_{D2}); 70.9 (C_2); 70.1 (C_5); 69.9 (C_3); 68.7 (C_7); 67.4 (C_4); 63.5 (C_6); 51.0 (C_8); 39.4, 39.2 (C_{D4},C_{D5}); 27.8 (C_{D3}); 27.0 (C_{D6}).

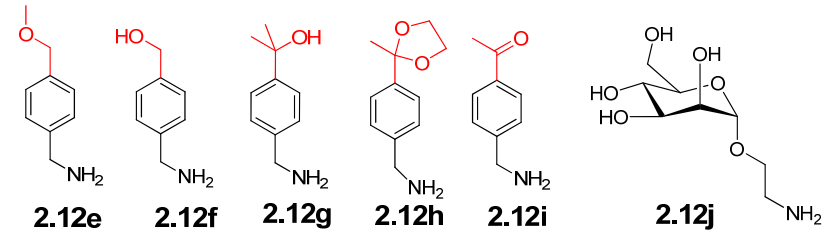
2.31 1H NMR
(400 MHz, $CDCl_3$)

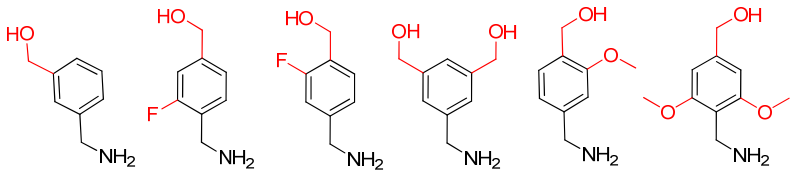


2.31 13C NMR
(100 MHz, $CDCl_3$)



2.4.4 Synthesis of amines 2.12e-j,n-s

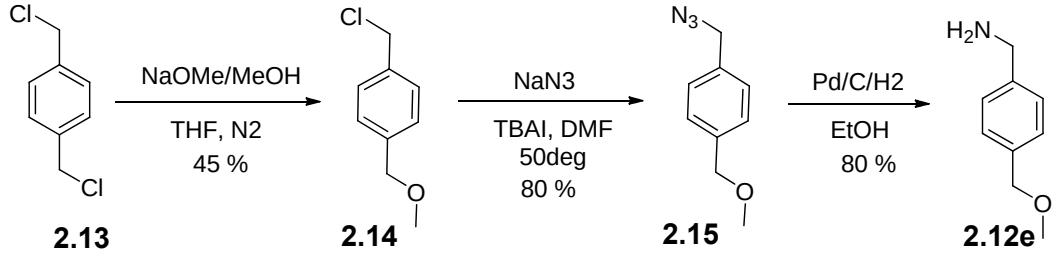




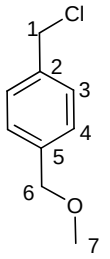
2.4.4.1 General procedure 1: reduction using $LiAlH_4$

To a 0.5 M solution of $LiAlH_4$ (2 eq per functional group) in dry THF a 0.5 M solution of starting material (1 eq) in dry THF was added at 0°C dropwise under nitrogen atmosphere. After the complete addition the reaction mixture was heated up to reflux for 3 h. The reaction was cooled to 0°C and worked up by addition of water (1 ml per 1g of $LiAlH_4$), 15% NaOH solution (1 ml per 1g of $LiAlH_4$) and water (3 ml per 1g of $LiAlH_4$) again. The precipitate was filtered off and washed with THF. The filtrate was concentrated under reduced pressure. The product was purified by flash chromatography (silica, DCM with gradient of methanol from 0 to 20%) or used without purification if the purity was sufficient.

2.4.4.2 (4-(methoxymethyl)phenyl)methanamine, 2.12e



1-(chloromethyl)-4-(methoxymethyl)benzene, 2.14[44]: To a solution of 1,4bis(chloromethyl)benzene **2.13** (200 mg, 1.14 mmol, 1.1 eq) in dry THF (1.3 ml) a 1 M solution of MeONa in methanol (0.9 ml, 1.01 mmol, 1 eq) was added dropwise at 0°C under nitrogen atmosphere. The reaction was stirred at room temperature for 16 h. The reaction was diluted with diethylether washed with water and brine, dried over sodium sulphate and concentrated under reduced pressure. The crude product was purified by flash chromatography (hexane with gradient of DCM from 20% to 50%) to yield 45% of pure product **2.14**.

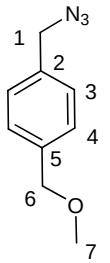


2.14

Yield: 45 %.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.39 (d, 2H, H_3, *J* = 8.2 Hz), 7.34 (d, 2H, H_4, *J* = 8.2 Hz), 4.60 (s, 2H, H_1), 4.48 (s, 2H, H_6), 3.41 (s, 3H, H_7).

1-(azidomethyl)-4-(methoxymethyl)benzene, 2.15: To a solution of **2.14** (65 mg, 0.38 mmol, 1 eq) in DMF (1.3 ml) NaN3 (198 mg, 3.05 mmol, 8 eq) and TBAI (14 mg, 0.04 mmol, 0.1 eq) was added. The reaction was heated up to 50°C and stirred for 16 h. The solvent was removed under reduced pressure and the residue was taken up with ether, washed with water and brine, dried over sodium sulphate and concentrated under reduced pressure. The crude product was purified by flash chromatography (hexane with gradient of DCM from 30 % to 70 %) to yield 80 % of pure product **2.15**.



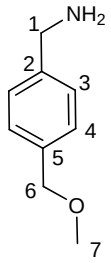
2.15

Yield: 80 %.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.35 (d, 2H, H_3, *J* = 8.1 Hz), 7.29 (d, 2H, H_4, *J* = 8.1 Hz), 4.45 (s, 2H, H_6), 4.31 (s, 2H, H_1), 3.38 (s, 3H, H_7)

^{13}C NMR (100 MHz, CDCl3): δ = 138.6 (C_2); 134.9 (C_5); 128.4, 128.2 (C_3, C_4); 74.4 (C_6); 58.3 (C_7); 54.7 (C_1).

(4-(methoxymethyl)phenyl)methanamine, 2.12e[45]: To a solution of **2.15** (50 mg, 0.28 mmol, 1 eq) in ethanol (9 ml) 10 % Pd/C was added in catalytic amount. The reaction was stirred under H_2 (1 atm) at room temperature for 2 h. The catalyst was filtered off through a celite pad. The filtrate was concentrated under reduced pressure to yield 80 % of pure product**.**



2.12e

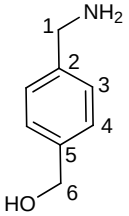
Yield: 80 %.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.30 - 7.24 (m, 4H, H_3, H_4), 4.41 (s, 2H, H_6), 3.83 (s, 2H, H_1), 3.35 (s, 3H, H_7).

^{13}C NMR (100 MHz, CDCl3): δ = 142.9 (C_2); 136.9 (C_5); 128.2, 127.3 (C_3, C_4); 74.6 (C_6); 58.2 (C_7); 46.4 (C_1).

2.4.4.3 (4-(aminomethyl)phenyl)methanol, 2.12f[46]

Using the general procedure 1 starting from methyl 4-(aminomethyl)benzoate hydrochloride

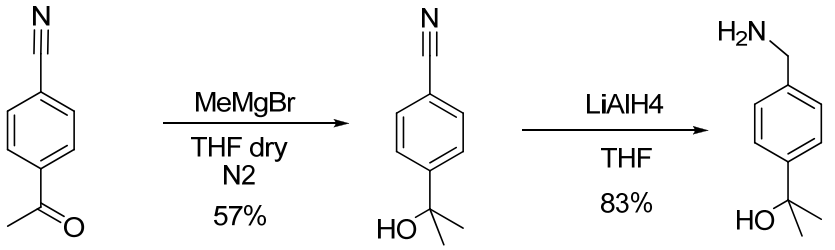


10h

Yield: 95 %.

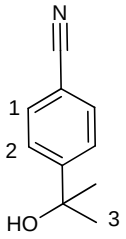
^{1}H NMR (400 MHz, CD_3OD): δ = 7.32 (s, 4H, H_3, H_4), 4.58 (s, 2H, H_6), 3.78 (s, 2H, H_1).

2.4.4.4 2-(4-(aminomethyl)phenyl)propan-2-ol, 2.12g



2.17 **2.18** **2.12g**

4-(2-hydroxypropan-2-yl)benzonitrile, 2.18[47]: To a solution of MeMgBr (3 M in Et_2O, 2.3 ml, 6.88 mmol, 5 eq) in THF (5 ml) a solution of 4-acetylbenzonitrile **2.17** (200 mg, 1.38 mmol, 1 eq) was added dropwise at 0°C under nitrogen atmosphere. The reaction was stirred for 2 h at room temperature, then cooled down to 0°C and quenched by addition of water. The reaction was diluted with ether, washed with water and brine. The organic phase was dried over sodium sulphate and concentrated under reduced pressure. The crude product was purified by flash chromatography (hexane with gradient of ethyl acetate from 10% - 30%) to yield pure product **2.18**.

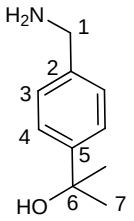


2.18

Yield: 67%

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.65 – 7.55 (m, 4 H, H_1, H_2), 1.57 (s, 6H, H_3).

2-(4-(aminomethyl)phenyl)propan-2-ol, 2.12g[47]: Using general procedure 1 starting from **2.12g**.



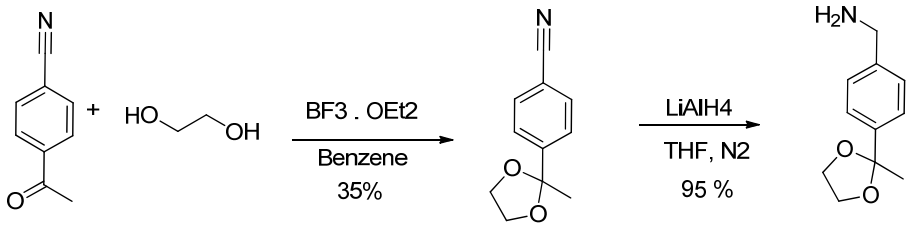
2.12g

Yield: 70 %.

^{1}H NMR (400 MHz, CD_3OD): δ = 7.46 (d, 2H, H_4, J = 8.4 Hz), 7.29 (d, 2H, H_3, J = 8.4 Hz), 3.79 (s, 2H, H_1), 1.52 (s, 6H, H_7).

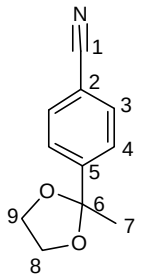
^{13}C NMR (100 MHz, CD_3OD): δ = 149.2 (C_2, C_5); 128.4 (C_3);, 126.0 (C_4); 73.0 (C_6); 47.3 (C_1); 32.1 (C_7).

2.4.4.5 (4-(2-methyl-1,3-dioxolan-2-yl)phenyl)methanamine, 2.12h



2.19 **2.20** **2.12h**

4-(2-methyl-1,3-dioxolan-2-yl)benzonitrile, 2.20[48]: To the solution of 4-acetylbenzonitrile **2.19** (300 mg, 2.06 mmol, 1 eq) in benzene (5 ml) under nitrogen atmosphere ethyleneglycol (0.35 ml, 6.20 mmol, 3 eq) was added in one portion and $BF_3.Et_3$ (0.08 ml, 0.62 mmol, 0.3 eq) dropwise. The solution was stirred at room temperature for 1 h then heated up to reflux for 5 h. The reaction was quenched by addition of triethylamine (few drops) and then diluted with diethyl ether. The mixture was transferred to a separatory funnel, washed with water and brine, dried over sodium sulphate and concentrated under reduced pressure. The crude was purified by flash chromatography (hexane : diethyl ether = 7 : 3) to yield 41 % of pure product.



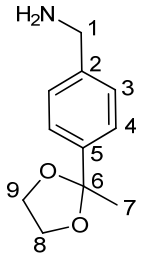
2.20

Yield: 41 %

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.64 – 7.56 (m, 4H, H_3, H_4), 4.09 – 3.99 (m, 2H, H_8, H_9), 3.78 – 3.68 (m, 2H, H_8, H_9), 1.61 (s, 3H, H_7).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 148.9 (C_5); 132.4 (C_3); 126.4 (C_4); 119.0 (C_1); 112.0 (C_2); 108.4 (C_6); 64.9 (C_8, C_9); 27.6 (C_7)

(4-(2-methyl-1,3-dioxolan-2-yl)phenyl)methanamine, 2.12h[49]: Using general procedure 1 starting from nitrile **2.20**.



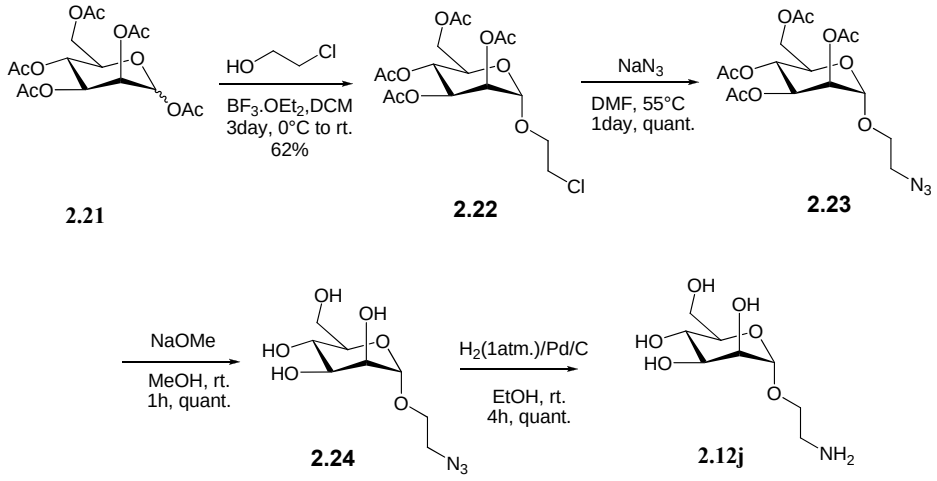
2.12h

Yield: 95 %.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.45 – 7.21 (m, 4 H, H_3, H_4), 4.06 – 3.96 (m, 2H, H_8, H_9), 3.85 (s, 2H, H_1), 3.80 – 3.70 (m, 2H, H_8, H_9), 1.63 (s, 3H, H_7).

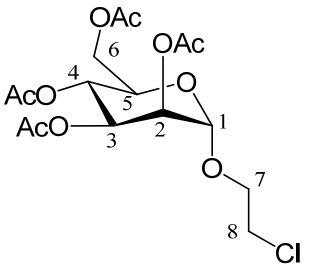
^{13}C NMR (100 MHz, $CDCl_3$): δ = 142.6, 142.0 (C_2, C_5); 127.2 (C_3); 125.7 (C_4); 109.0 (C_6); 64.6 (C_8, C_9); 46.3 (C_1); 27.8 (C_7).

2.4.4.6 (2-aminoethyl)-α-D-mannopyranoside, 2.12j



***(2-chloroethyl)-2,3,4,6-tetraacetyl-α-D*-mannopyranoside, 2.22**

To a solution of the peracetylated mannose (100 mg, 0.256 mmol, 1 eq.) in dry DCM (8.5 mL) at room temperature under nitrogen atmosphere chloroethanol (22 µl, 0.333 mmol, 1.3 eq.) was added. The solution was cooled at 0°C and $BF_3 – Et_2O$ (126 µl, 1.025 mmol, 4 eq.) was added. The reaction was kept at 0°C for 30 minutes, then warmed at room temperature and stirred overnight. After completion (TLC 6:4 Hex:EtOAc) the reaction mixture was diluted with DCM, poured into a ice-water mixture and extracted twice with DCM. Organic phases were collected, washed with sat. $NaHCO_3$ and dried over sodium sulphate. The solvent was evaporated at reduced pressure obtaining 108 mg of crude that was purified by flash chromatography (silica, Toluene:EtOAc = 7:3) leading to 63 mg of pure product.



2.22

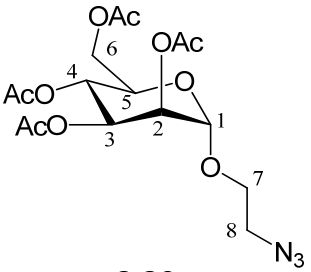
Yield: 60%.

$[\alpha]^{20}_{D}$= (c = 0.47, chloroform).

MS (FAB) calculated for $[C_{16}H_{23}ClO_{10}Na]^{+}$: 433 ; found: 433.

^{1}H NMR (400 MHz, $CDCl_3$): 5.33 (dd, 1H, H_3, $J_{3\text{-}4}$ =10.0 Hz, $J_{3\text{-}2}$ = 3.4 Hz), 5.30 - 5.23 (m, 2H, H_2, H_4), 4.85 (d, 1H, H_1, $J_{1\text{-}2}$ = 1.3 Hz), 4.25 (dd, 1H, H_{6B}, J_{gem} = 12.4 Hz, $J_{6B\text{-}5}$ = 5.5 Hz), 4.15 - 4.06 (m, 2H, H_{6A}, H_5), 3.95 - 3.86 (m, 1H, H_{7B}), 3.84 - 3.76 (m, 1H, H_{7A}), 3.66 (t, 2H, H_8, J = 5.7 Hz), 2.14 (s, 3H, CH_3CO), 2.08 (s, 3H, CH_3CO), 2.03 (s, 3H, CH_3CO), 1.98 (s, 3H, CH_3CO).
^{13}C NMR (100 MHz, $CDCl_3$): 170.8 (**C**OCH3); 170.2 (**C**OCH3); 170.1 (**C**OCH3); 170.0 (**C**OCH3); 98.1 (C_1); 69.7 (C_2); 69.2 (C_3); 69.2 (C_5); 68.9 (C_7); 66.3 (C_4); 62.7 (C_6); 42.6 (C_8); 21.1 (**C**H3CO); 21.0 (**C**H3CO); 20.9 (**C**H3CO); 20.9 (**C**H3CO).

(2-azidoethyl)-2,3,4,6-tetraacetyl-α-D-mannopyranoside, 2.23: To a solution of the chloroethyl derivative **2.22** (43.0 mg, 0.105 mmol, 1 eq.) in dry DMF (0.26 ml) under nitrogen atmosphere sodium azide (34 mg, 0.523 mmol, 5 eq.) was added. The reaction was warmed at 50°C and stirred at that temperature for 1 day. After completion (1H-NMR) the reaction mixture was diluted with EtOAc, washed with water. Aqueous phase was extracted again with EtOAc, the organic phases were collected, dried over sodium sulphate and the solvent was evaporated at reduced pressure, obtaining 43 mg of pure product as a colourless oil.



2.23

Yield: quantitative.

$[\alpha]^{20}_{D}$= + 31.5 (c: 0.35, $CHCl_3$).

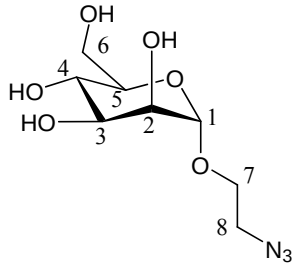
MS (FAB) calculated for $[C_{16}H_{24}N_3O_{10}]$+: 418; found: 418.

calculated for $[C_{16}H_{23}N_3O_{10}Na]$+: 440; found: 440.

^{1}H NMR (400 MHz, $CDCl_3$): 5.34 (dd, 1H, H_3, $J_{3\text{-}4}$ =10.0 Hz, $J_{3\text{-}2}$ = 3.2 Hz), 5.31 - 5.24 (m, 2H, H_2, H_4), 4.85 (d, 1H, H_1, $J_{1\text{-}2}$ = 1.2 Hz), 4.27 (dd, 1H, H_{6B}, J_{gem} = 12.0 Hz, $J_{6B\text{-}5}$ = 5.2 Hz), 4.11 (dd, 1H, H_{6A}, $J_{6A\text{-}5}$ = 2.4 Hz), 4.06 - 4.00 (m, 1H, H_5), 3.88 - 3.81 (m, 1H, H_{7B}), 3.69 - 3.62 (m, 1H,
H_{7A}), 3.51 - 3.38 (m, 2H, H_8), 2.14 (s, 3H, CH_3CO), 2.08 (s, 3H, CH_3CO), 2.03 (s, 3H, CH_3CO), 1.97 (s, 3H, CH_3CO).

^{13}C NMR (100 MHz, $CDCl_3$): 170.8 (**C**OCH_3); 170.2 (**C**OCH_3); 170.0 (**C**OCH_3); 170.0 (**C**OCH_3); 98.0 (C_1); 69.6 (C_2); 69.1 (C_3); 69.1 (C_5); 67.3 (C_7); 66.3 (C_4); 62.7 (C_6); 50.6 (C_8); 21.1 (**C**H_3CO); 21.0 (**C**H_3CO); 20.9 (**C**H_3CO); 20.9 (**C**H_3CO).

***(2-azidoethyl)-α-D-mannopyranosid, 2.24**:* To a solution of the azidoethyl derivative **2.23** (3.26 g, 7.81 mmol, 1 eq) in dry MeOH (53 ml), freshly prepared MeONa 1M in dry MeOH (1.25 ml) was added. The reaction was stirred at room temperature, monitoring by TLC (Hex:EtOAc = 1:1). After 20 min the reaction mixture was neutralized with an acidic resin (Amberlite IRA 120 H+) and the solvent was evaporated at reduced pressure, obtaining 1.95 g of the azidoethylmannoside **2.24**, as a white solid, which was used without any other purification.



2.24

Yield = quantitative.

$[\alpha]^{D}_{20}$ = +55.64 (c: 1.2, MeOH).

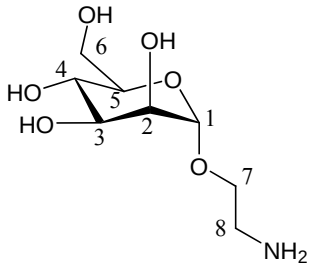
MS (ESI) calculated for $[C_8H_{15}N_3O_6Na]^+$: 272.1; found: 272.6
calculated for $[C_8H_{15}N_3O_6K]^+$: 289.1; found: 288.4.

^{1}H NMR (400 MHz, D_2O): 4.95 (d, 1H, H_1, $J_{1\text{-}2}$= 1.6 Hz), 4.01 (dd, 1H, H_2, $J_{2\text{-}3}$= 3.2 Hz), 3.98 - 3.92 (m, 1H, H_{7B}), 3.95 - 3-91 (m, 1H, H_{6B}), 3.87 (dd, 1H, H_3, $J_{3\text{-}4}$= 9.4 Hz), 3.82 – 3.77 (m, 1H, H_{6A}), 3.78-3.73 (m, 1H, H_{7A}), 3.72-3.68 (m, 2H, H_4, H_5), 3.58 (ddd, 1H, H8B, J_{gem}= 13.6 Hz, $J_{8B\text{-}7}$= 6.4 Hz, $J_{8B\text{-}7}$' = 3.2 Hz), 3.52 (ddd, 1H, H_{8A}, $J_{8A\text{-}7}$ = 6.0 Hz, $J_{8A\text{-}7'}$ = 3.2 Hz).

^{13}C NMR (100 MHz, D_2O): 99.6 (C_1); 72.7 (C_4); 70.2 (C_3); 69.7 (C_2); 66.5 (C_5); 66.1 (C_7); 60.7

(C_6); 50.0 (C_8).

***(2-aminoethyl)-α-D-mannopyranoside, 2.12j*:** To a solution of the azide **2.24** (20 mg, 0.08 mmol, 1 eq) in EtOH (4 ml) a catalytic amount of Pd on carbon was added. The suspension was stirred under hydrogen atmosphere at room temperature. After completition (2 h, TLC $CHCl_3$:MeOH = 8:2) the reaction mixture was filtered over a celite pad and the solvent was evaporated at reduced pressure, obtaining 17 mg of pure product.



2.12j

Yield = 95%

MS (ESI) calculated for $[C_8H_{18}O_6]^+$: 224; found: 224

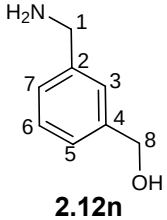
^{1}H NMR (400 MHz, CD_3OD): 4.81 (s, 1H, H_1), 4.02 – 3.78 (m, 3H, H_2, H_{6A}, H_{7A}) 3.75 – 3.56 (m, 4H, H_3, H_{6B}, H_{7B}, H_4), 3.55 – 3.49 (m, 1H, H_5), 3.15 – 3.06 (m, 2H, H_8).

^{13}C NMR (100 MHz, D_2O): 102.1 (C_1); 75.3 (C_5); 72.6 (C_3); 71..9 (C_2); 68.7 (C_4); 66.0 (C_7); 63.1

(C_6); 41.0 (C_8).

2.4.4.7 (3-(aminomethyl)phenyl)methanol 2.12n[50]

Using general procedure 1 starting from 3-formylbenzonitrile. yield: 92%.



2.12n

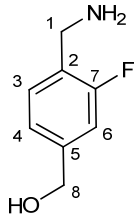
Yield: 92%.

^{1}H NMR (400 MHz, CD_3OD): δ = 7.41 – 7.28 (m, 4 H, H_3, H_5, H_6, H_7), 4.68 (s, 2H, H_8), 3.85 (s, 2H, H_1).

^{13}C NMR (100 MHz, CD_3OD): δ = 143.9, 143.2 (C_2, C_4); 129.7(C_3); 127.4, 127.1, 126.7 (C_5, C_6, C_7);, 65.3 (C_8); 46.8 (C_1).

2.4.4.8 (4-(aminomethyl)-3-fluorophenyl)methanol 2.12o

Using general procedure 1 starting from 2-fluoro-4-formylbenzonitrile.



2.12o

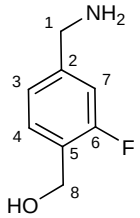
Yield: 53 %.

^{1}H NMR (400 MHz, CD_3OD): δ = 7.40 (t, 1H, H_3, $J_{7\text{-}6} = J_{3\text{-}F} = 7.8$ Hz), 7.19 - 7.09 (m, 2H, H_6, H_4), 4.63 (s, 2H, H_8), 3.86 (s, 2H, H_1).

^{13}C NMR (100 MHz, CD_3OD): δ = 162.4 (d, C_3, $J_{C3\text{-}F} = 244$ Hz); 144.7 (d, C_5, $J_{C5\text{-}F} = 7.2$ Hz); 130.7 (d, C_7, $J_{C7\text{-}F} = 5.0$ Hz); 129.17 (d, C_2, $J_{C2\text{-}F} = 15.5$ Hz); 123.7 (d, C_4, $J_{C4\text{-}F} = 3.22$ Hz); 114.5 (d, C_6, $J_{C6\text{-}F} = 22.4$ Hz); 64.4 (d, C_8, $J_{C8\text{-}F} = 1.6$ Hz); 40.3 (d, C_1, $J_{C1\text{-}F} = 3.9$ Hz).

2.4.4.9 (4-(aminomethyl)-2-fluorophenyl)methanol 2.12p

Using general procedure 1 starting from 4-cyano-2-fluorobenzoic acid; yield: 70 %.



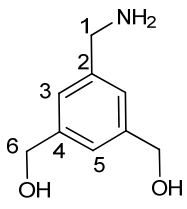
2.12p

^{1}H NMR (400 MHz, CD_3OD): δ = 7.44 (t, 1H, H_4, $J_{4\text{-}3} = J_{4\text{-}F} = 7.8$ Hz), 7.19 - 7.09 (m, 2H, H_3, H_7), 4.68 (s, 2H, H_8), 3.81 (s, 2H, H_1).

^{13}C NMR (100 MHz, CD_3OD): δ = 162.1 (d, C_6, $J_{C6\text{-}F} = 245$ Hz); 145.8 (d, C_2, $J_{C2\text{-}F} = 7.1$ Hz); 130.7 (d, C_4, $J_{C4\text{-}F} = 4.9$ Hz); 128.1 (d, C_5, $J_{C5\text{-}F} = 15.2$ Hz); 124.2 (d, C_3, $J_{C3\text{-}F} = 3.16$ Hz); 115.0 (d, C_7, $J_{C7\text{-}F} = 22.0$ Hz); 58.8 (d, C_8, $J_{C8\text{-}F} = 4.4$ Hz); 46.2 (d, C_1, $J_{C1\text{-}F} = 1.5$ Hz).

2.4.4.10 (5-(aminomethyl)-1,3-phenylene)dimethanol 2.12q

Using general procedure 1 starting from 5-cyanoisophthalic acid.



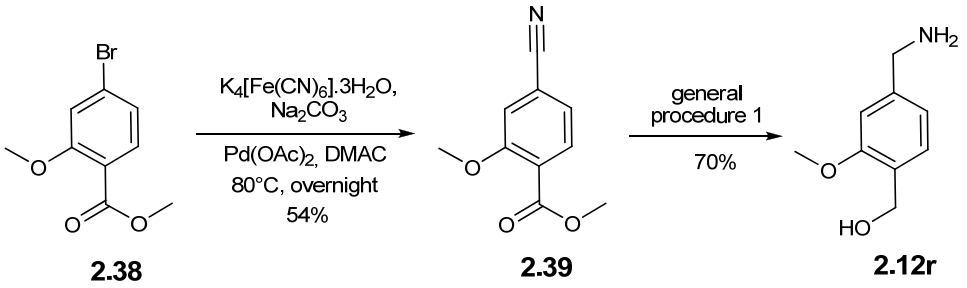
2.12q

Yield: 35 %.

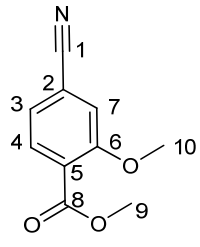
^{1}H NMR (400 MHz, CD_3OD): δ = 7.26 (s, 1H, H_5), 7.23 (s, 2H, H_3), 4.62 (s, 4H, H_6), 3.88 (s, 2H, H_1).

^{13}C NMR (100 MHz, CD_3OD): δ = 143.6 (C_4); 141.5 (C_2); 126.4 (C_3); 125.9 (C_5); 65.2 (C_6); 46.1 (C_1).

2.4.4.11 (4-(aminomethyl)-2-methoxyphenyl)methanol 2.12r



methyl 4-cyano-2-methoxybenzoate 2.39: A flask charged with methyl 4-bromo-2-methoxybenzoate **2.38** (200 mg, 0.82 mmol, 1 eq), $K_4[Fe(CN)_6].3H_2O$ (76 mg, 0.18 mmol, 0.22 eq), Na_2CO_3 (87 mg, 0.82 mmol, 1 eq), $Pd(OAc)_2$ (1 mg, 0.04 mmol, 0.05 eq) was degassed and nitrogen atmosphere was introduced (3 cycles), then the reagents were dissolved by addition of dry DMAC (1.4 ml). The reaction mixture was heated to 80°C and stirred under nitrogen atmosphere for 24 h. The TLC (hexane : AcOEt = 8:2), indicated presence of starting material so another portion of $K_4[Fe(CN)_6].3H_2O$ (172 mg, 0.408 mmol, 0.5 eq), Na_2CO_3 (87 eq, 0.82 mmol, 1 eq), $Pd(OAc)_2$ (1 mg, 0.04 mmol, 0.05 eq), and 1 mL of DMAC was added. The reaction mixture was stirred for additional 24 h at 70°C then cooled down to room temperature, diluted with ethyl acetate and filtered through a short silica pad. The filtrate was concentrated under reduced pressure and the crude residue was purified by flash chromatography (hexane : ethylacetate = 9 : 1) to yield pure product **2.39**.



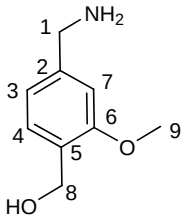
2.39

yield: 54 %

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.83 (d, 1H, H_4, $J_{4\text{-}3}$ = 7.9 Hz), 7.29 (dd, 1H, H_3, $J_{3\text{-}4}$ = 7.9 Hz, $J_{3\text{-}7}$ = 1.3 Hz), 7.26 (d, 1H, H_7, $J_{7\text{-}3}$ = 1.3 Hz), 3.95 (s, 3H, H_{10}) 3.93 (s, 1H, H_9).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.3 (C_8); 159.0 (C_2); 132.2 (C_4); 124.8 (C_5); 124.0 (C_3); 118.0 (C_1); 116.6 (C_6); 115.4 (C_7); 56.3 (C_9); 52.7 (C_{10}).

(4-(aminomethyl)-2-methoxyphenyl)methanol 2.12r: Using the general procedure 1 starting from **2.39.**



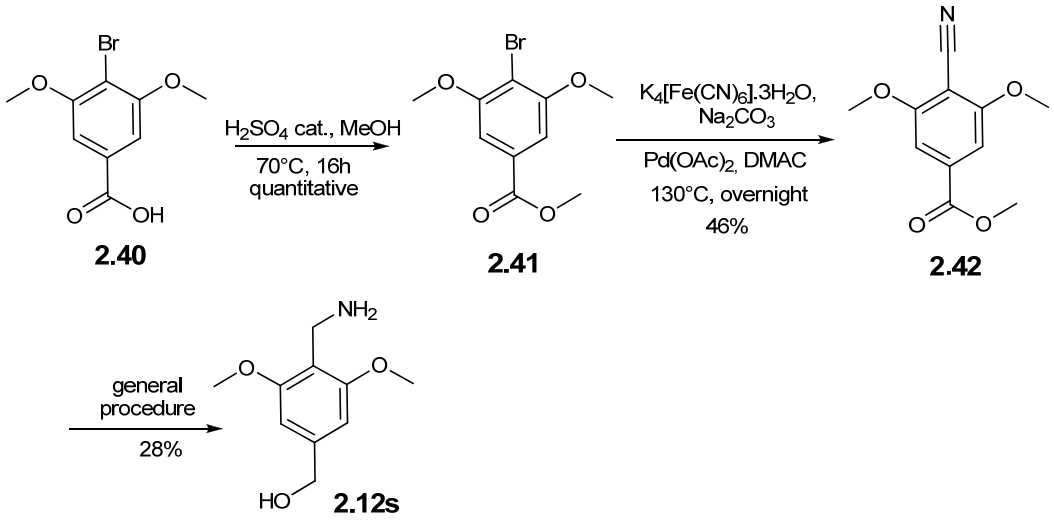
2.12r

Yield: 70 %.

^{1}H NMR (400 MHz, CD_3OD): δ = 7.30 (d, 1H, H_4, $J_{4\text{-}3}$ = 7.6 Hz), 6.96 (s, 1H, H_7), 6.91 (d, 1H, H_3, $J_{3\text{-}4}$ = 7.6 Hz), 4.60 (s, 2H, H_8) 3.88 (s, 3H, H_9), 3.82 (s, 2H, H_1).

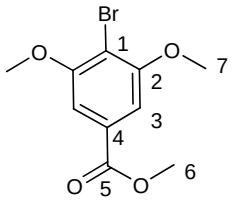
^{13}C NMR (100 MHz, CD_3OD): δ = 158.8 (C_6); 143.8 (C_2); 129.6 (C_5); 129.4 (C_4); 120.5 (C_3); 110.6 (C_7); 60.5 (C_8); 56.1 (C_9); 46.8 (C_1).

2.4.4.12 4-(aminomethyl)-3,5-dimethoxyphenyl)methanol 2.12s



2.40 **2.41** **2.42** **2.12s**

methyl 4-bromo-3,5-dimethoxybenzoate 2.41: To a solution of 4-bromo-3,5-dimethoxybenzoic acid **2.40** (1,00 g, 3.83 mmol, 1 eq) in methanol (4 ml) H_2SO_4 (25 µL, 0.46 mmol, 0.12 eq).was added. The reaction was stirred under reflux for 16 h. The solvent was removed under reduced pressure and the crude residue was dissolved in DCM, washed with saturated $NaHCO_3$ solution and water. The organic phase was dried over sodium sulphate and concentrated under reduced pressure to afford the pure product **2.41.**



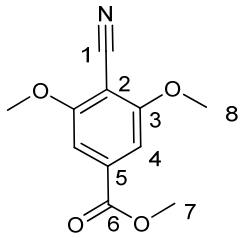
2.41

Yield: quantitative

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.25 (s, 2H, H_3), 3.96 (s, 6H, H_7), 3.94 (s, 3H, H_6).

^{13}C NMR (100 MHz, $CDCl_3$): δ 166.8 (C_5); 157.3 (C_2); 130.2 (C_4); 107.0 (C_1); 105.6 (C_3); 56.9 (C_7); 52.6 (C_6).

methyl 4-cyano-3,5-dimethoxybenzoate 2.42: A flask charged with **2.41** (200 mg, 0.73 mmol, 1 eq), $K_4[Fe(CN)_6].3H_2O$ (307 mg, 0.73 mmol, 1 eq), Na_2CO_3 (77 mg, 0.727 mmol, 1 eq) and $Pd(OAc)_2$ (3-5%) was degassed and nitrogen atmosphere was introduced (3 cycle), then the reagents were dissolved by addition of dry DMAC (1 ml). The reaction mixture was heated up to 130°C and stirred under nitrogen atmosphere for 24 h. The reaction was cooled to room temperature, diluted with ethyl acetate and filtered through a short silica pad. The filtrate was concentrated under reduced pressure and the crude residue was purified by flash chromatography (hexane : ethylacetate = 9 : 1) to yield pure product **2.42**.



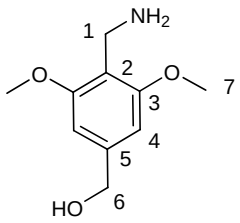
2.42

Yield: 46 %

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.22 (s, 2H, H_4), 3.98 (s, 6H, H_8), 3.96 (s, 3H, H_7).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.4 (C_6); 162.6 (C_3); 136.1 (C_5); 113.6 (C_1); 105.0 (C_4); 56.9 (C_8); 53.2 (C_7).

(4-(aminomethyl)-3,5-dimethoxyphenyl)methanol 2.12s: Using the general procedure 1 starting from **2.42.**



2.12s

Yield: 28 %.

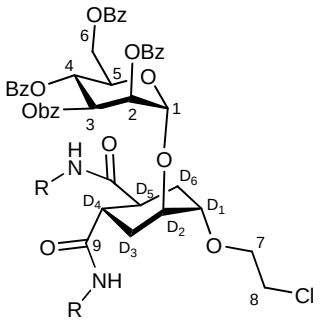
^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.66 (s, 2H, H_4), 4.61 (s, 2H, H_6), 3.93 (s, 2H, H_1), 3.85 (s, 6H, H_7).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 159.8 (C_3); 145.4 (C_5); 113.8 (C_2); 103.0 (C_4); 65.4 (C_6); 56.6 (C_7) 34.1 (C_1).

2.4.5 Synthesis and characterization of 1,2-Cyclohexanedicarboxamides 4-(2-chloroethoxy)-5-[(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)oxy]-, (1*S*,2*S*,4*S*,5*S*), 2.10a-j

2.4.5.1 General procedure 2

Amine **2.12** (3 eq) was added to a 0.1 M PFP-scaffold **2.9** (1 eq) in dry THF under stirring and under nitrogen atmosphere at room temperature. After completion (1-12 h, checked by TLC, hex : EtOAc) the solvent was evaporated under reduced pressure. The crude was purified by flash chromatography (hexane with gradient of ethyl acetate from 30% to 80%)

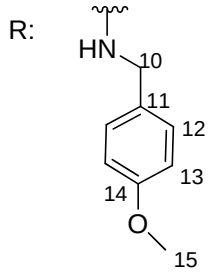


*General structure and numbering of pseudobimannoside bis-amides **2.10a-j** in the NMR characterizations*

2.4.5.2 *N*^{1},*N*^{2}-bis(4-methoxybenzyl)amide, 2.10a

1,2-Cyclohexanedicarboxamides-N^1,N^2-bis(4-methoxybenzyl)-4-(2-chloroethoxy)-5-[(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)oxy]- (1*S*,2*S*,4*S*,5*S*)

Prepared according to general procedure 2, using amine **2.12a.**



2.10a

Yield: 90%

$[\alpha]^{20}_{D}$ = -35.62 (c = 0.45; $CHCl_3$)

MS (FAB): calculated for $[C_{60}H_{60}ClN_2O_{15}]^+$: 1083; found: 1083

calculated for $[C_{60}H_{60}ClN_2O_{15}Na]^+$: 1105; found: 1105

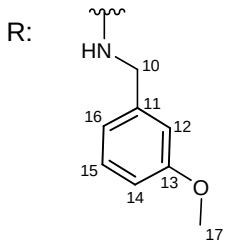
^{1}H NMR (400 MHz, $CDCl_3$): 8.07 (d, 2H, H_{BZ}, *J* = 7.2 Hz), 8.02 (d, 2H, H_{BZ}, *J* = 7.2 Hz), 7.97 (d, 2H, H_{BZ}, *J* = 7.2 Hz), 7.73 (d, 2H, H_{BZ}, *J* = 7.2 Hz), 7.64-7.49 (m, 3H, H_{BZ}), 7.48-7.35 (m, 7H, H_{BZ}), 7.25-7.16 (m, 6H, H_{BZ}, H_{12}), 6.91-6.71 (m, 5H, H_{13}, NH), 6.17 (brs, 1H, NH), 6.13 (dd, 1H, H_4, $J_{4\text{-}5}$ = 10.4 Hz, $J_{4\text{-}3}$ = 8.0 Hz), 5.88 (dd, 1H, H_3, $J_{3\text{-}2}$ = 3.2 Hz, $J_{3\text{-}4}$ = 10.4 Hz), 5.86-5.79 (m, 1H, H_8), 5.66 (dd, 1H, H_2, $J_{2\text{-}1}$ = 1.6 Hz, $J_{2\text{-}3}$ = 3.2 Hz), 5.26 (d, 1H, H_1, $J_{1\text{-}2}$ = 1.6 Hz), 4.73-4.65 (m, 1H, H_{6b}), 4.55-4.46 (m, 2H, H_5, H_{6a}), 4.43-4.32 (m, 2H, H_{10a}), 4.30-4.19 (m, 2H, H_{10b}), 4.07 (m, 1H, D_2), 3.79 (s, 3H, H_{15}), 3.77 (m, 1H, D_1), 3.73 (s, 3H, H_{15}), 3.71-3.60 (m, 2H, $H_{7a,b}$), 3.57-3.51 (m, 2H, $H_{8a,b}$) 2.97-2.85 (m, 2H, D_4, D_5), 2.33-2.12 (m, 2H, D_{3eq}, D_{6eq}), 2.09-1.94 (m, 2H, D_{3ax}, D_{6ax}).

^{13}C NMR (100 MHz, $CDCl_3$): 174.2, 174.0 (**C**$_9$); 166.2, 165.9, 165.9, 165.8 (**C**O_{BZ}); 159.1, 159.0 (C_{14}); 133.8, 133.7, 133.4, 133.3 (**C**H_{BZ}); 130.7, 130.4, 130.0, 129.0, 129.0 (**C**$_{quatBZ}$, C_{11}); 130.1, 130.0, 129.9, 129.2, 128.8, 128.6, 128.6, 128.5 (**C**H_{BZ}, C_{12}); 114.2, 114.2 (C_{13}); 97.1 (C_1); 75.3 (C_{D1}); 74.5 (C_{D2}); 71.6 (C_2); 70.3 (C_3); 70.1 (C_5); 69.7 (C_7); 66.8 (C_4); 63.1 (C_6); 55.4, 55.4 (C_{15}); 43.3 (C_8) 43.2, 43.1 (C_{10}); 41.8, 41.1 (C_{D4}, C_{D5}); 28.8, 28.4 (C_{D3}, C_{D6}).

2.4.5.3 N^1,N^2-bis(3-methoxybenzyl)amide, 2.10b

1,2-Cyclohexanedicarboxamides-N^1,N^2-bis(3-methoxybenzyl)-4-(2-chloroethoxy)-5-[(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)oxy]- (1*S*,2*S*,4*S*,5*S*)

Prepared according to general procedure 2, using amine **2.12b.**



2.10b

Yield: 96%

MS (FAB): calculated for $[C_{60}H_{60}ClN_2O_{15}]^+$: 1083; found: 1083

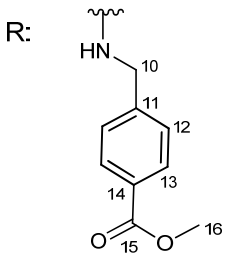
calculated for $[C_{60}H_{60}ClN_2O_{15}Na]^+$: 1105; found: 1105

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.06 – 7.90 (m, 6H, H_{Bz}), 7.71 – 7.65 (m, 2H, H_{Bz}), 7.60 – 7.46 (m, 3H, H_{Bz}), 7.43–7.32 (m, 7H, H_{Bz}), 7.25 – 7.13 (m, 4H, H_{Bz}, H_{15}), 6.88 - 6.67 (m, 7H, H_{12}, H_{14}, H_{16}, H_{NH}), 6.17 (t, 1H, H_{NH}, $J_{NH\text{-}10}$ = 5.8 Hz), 6.10 (t, 1H, H_4, $J_{4\text{-}3}$ = $J_{4\text{-}5}$= 10.0 Hz), 5.85 (dd, 1H, H_3, $J_{3\text{-}4}$ = 10.0 Hz, $J_{3\text{-}2}$= 3.3), 5.66 (brs, 1H, H_2), 5.23 (brs, 1H, H_1), 4.70 – 4.64 (m, 1H, H_{6a}), 4.51 – 4.30 (m, 4H, H_{6b}, H_{10a}, H_5), 4.30-4.19 (m, 2H, H_{10b}), 4.04 (m, 1H, D_2), 4.75 (s, 3H, H_{17}), 3.69 (s, 3H, H_{17}), 3.67 – 3.55 (m, 3H, D_1, $H_{7a,b}$), 3.53 - 3.45 (m, 2H, H_8), 3.01 – 2.87 (m, 2H, D_4, D_5), 2.26 - 2.10 (m, 2H, D_{3eq}, D_{6eq}), 2.07 - 1.95 (m, 2H, D_{3ax}, D_{6ax}).

2.4.5.4 N^1,N^2-bis(4-carbomethoxybenzyl)amide, 2.10c

1,2-Cyclohexanedicarboxamides-N^1,N^2-bis(4-carbomethoxybenzyl)-4-(2-chloroethoxy)-5-[(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)oxy]- (1*S*,2*S*,4*S*,5*S*)

Prepared according to general procedure 2, using amine **2.12c.**



2.10c

Yield: 63%

MS (ESI): calculated for $[C_{62}H_{59}ClN_2O_{17}Na]^+$: 1162.6; found: 1162.6

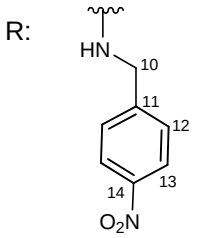
^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.06 – 8.03 (m, 2H, H_{Bz}), 7.99 – 7.86 (m, 10H, H_{Bz}, H_{13}), 7.68 – 7.65 (m, 2H, H_{Bz}), 7.59 – 7.51 (m, 2H, H_{Bz}), 7.50–7.45 (m, 1H, H_{Bz}), 7.41 – 7.26 (m, 8H, H_{Bz}, H_{12}), 7.22 – 7.17 (m, 2H, H_{Bz}), 7.06 (t, 1H, H_{NH}, $J_{NH\text{-}10}$ = 5.62 Hz), 6.41 (t, 1H, H_{NH}, $J_{NH\text{-}10}$ = 5.8 Hz), 6.11 (t, 1H, H_4, $J_{4\text{-}3}$ = $J_{4\text{-}5}$= 10.0 Hz), 5.87 (dd, 1H, H_3, $J_{3\text{-}4}$ = 10.0 Hz, $J_{3\text{-}2}$= 3.3), 5.66 (dd, 1H, H_2, $J_{2\text{-}1}$ = 1.7 Hz, $J_{2\text{-}3}$= 3.3 Hz), 5.24 (d, 1H, H_1, $J_{1\text{-}2}$= 1.7 Hz), 4.72 – 4.62 (m, 1H, H_{6a}), 4.51 – 4.30 (m, 6H, H_{6b}, $H_{10a,b}$, H_5), 4.09 - 4.04 (m, 1H, D_2), 4.87 (s, 3H, H_{16}), 3.84 (s, 3H, H_{16}), 3.78 – 3.74 (m, 1H, D_1), 3.71 - 3.58 (m, 2H, $H_{7a,b}$), 3.53 - 3.45 (m, 2H, H_8), 3.08 – 2.98 (m, 2H, D_4, D_5), 2.26 - 2.10 (m, 2H, D_{3eq}, D_{6eq}), 2.07 - 1.95 (m, 2H, D_{3ax}, D_{6ax}).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.6, 174.5 (C_9); 167.0, 167.0 (C_{15}); 166.3, 166.1, 166.0, 165.7 (CO_{BZ}); 143.8, 143.6 (C_{11}); 133.9, 133.7, 133.6, 133.4 (CH_{BZ}); 130.1, 130.1, 129.9, 129.9 (CH_{BZ}); 129.3, 129.3, 129.1, 128.9 ($Cquat_{BZ}$, C_{14}); 128.8, 128.7, 128.7, 128.5 (CH_{BZ}); 127.6, 127.5 (C_{12}, C_{13}); 97.1 (C_1); 75.3 (C_{D1}); 74.4 (C_{D2}); 72.9 (C_2); 71.5 (C_3); 70.4 (C_5); 69.9 (C_7); 66.7 (C_4); 63.0 (C_6); 52.3, 52.2 (C_{16}); 43.4 (C_8); 43.3, 43.2, (C_{10}); 41.7, 41.0 (C_{D4}, C_{D5}); 29.0 (C_{D3}); 28.6 (C_{D6}).

2.4.5.5 N^1,N^2-bis(4-nitrobenzyl)amide, 2.10d

1,2-Cyclohexanedicarboxamides-N^1,N^2-bis(4-nitrobenzyl)-4-(2-chloroethoxy)-5-[(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)oxy]- (1*S*,2*S*,4*S*,5*S*)

Prepared according to general procedure 2, using amine **2.12d.**



2.10d

Yield: 91%

MS (FAB): calculated for $[C_{58}H_{53}ClN_4O_{17}]^+$: 1013; found: 1013

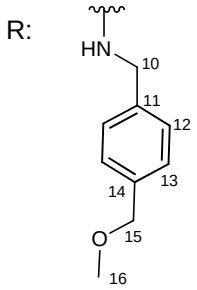
calculated for $[C_{58}H_{53}ClN_4O_{17}Na]^+$: 1135; found: 1135

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.14 – 7.90 (m, 10H, H_{Bz}, H_{13}), 7.68 – 7.64 (m, 2H, H_{Bz}, H_{13}), 7.61 – 7.47 (m, 3H, H_{Bz}), 7.43 – 7.31 (m, 10H, H_{13}, H_{Bz}), 7.26–7.11 (m, 4H, H_{NH}, H_{Bz}), 6.52 (t, 1H, H_{NH}, $J_{NH\text{-}10}$ = 6.1 Hz), 6.14 (t, 1H, H_4, $J_{4\text{-}3}$ = $J_{4\text{-}5}$= 10.0 Hz), 5.84 (dd, 1H, H_3, $J_{3\text{-}4}$ = 10.0 Hz, $J_{3\text{-}2}$= 3.3), 5.68 (dd, 1H, H_2, $J_{2\text{-}1}$ = 1.7 Hz, $J_{2\text{-}3}$= 3.3 Hz), 5.25 (d, 1H, H_1, $J_{1\text{-}2}$= 1.7 Hz), 4.72 – 4.62 (m, 1H, H_{6a}), 4.60 – 4.35 (m, 6H, H_{6b}, $H_{10a,b}$, H_5), 4.11 - 4.07 (m, 1H, D_2), 3.78 – 3.74 (m, 1H, D_1), 3.71 - 3.66 (m, 2H, $H_{7a,b}$), 3.56 - 3.52 (m, 2H, H_8), 3.15 – 2.98 (m, 2H, D_4, D_5), 2.26 - 2.00 (m, 4H, D_3, D_6).

2.4.5.6 N^1,N^2-bis(4-(methoxymethyl)benzyl)amide 2.10e

1,2-Cyclohexanedicarboxamides-N^1,N^2-bis(4-(methoxymethyl)benzyl)-4-(2-chloroethoxy)-5-[(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)oxy]- (1*S*,2*S*,4*S*,5*S*)

Prepared according to general procedure 2, using amine **2.12e.**



2.10e

Yield: 91%

MS (ESI): calculated for $[C_{62}H_{63}ClN_2O_{15}Na]^+$: 1134.6; found: 1134.7

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.06 – 7.92 (m, 6H, H_{Bz}), 7.72 – 7.68 (m, 2H, H_{Bz}), 7.60 – 7.47 (m, 3H, H_{Bz}), 7.42 – 7.32 (m, 7H, H_{13}, H_{Bz}), 7.29 – 7.19 (m, 10H, H_{13}, H_{Bz}), 6.86 (t, 1H, H_{NH}, $J_{NH\text{-}10}$ = 5.7 Hz), 6.34 (t, 1H, H_{NH}, $J_{NH\text{-}10}$ = 5.7 Hz), 6.19 (t, 1H, H_4, $J_{4\text{-}3}$ = $J_{4\text{-}5}$= 10.0 Hz), 5.92 (dd, 1H, H_3, $J_{3\text{-}4}$ = 10.0 Hz, $J_{3\text{-}2}$= 3.3), 5.64 (dd, 1H, H_2, $J_{2\text{-}1}$ = 1.7 Hz, $J_{2\text{-}3}$= 3.3 Hz), 5.24 (d, 1H, H_1, $J_{1\text{-}2}$= 1.7 Hz), 4.70 – 4.63 (m, 1H, H_{6a}), 4.51 – 4.20 (m, 10H, H_{15}, H_{6b}, $H_{10a,b}$, H_5), 4.08 - 4.04 (m, 1H, D_2), 3.78 – 3.74 (m, 1H, D_1), 3.71 - 3.58 (m, 2H, $H_{7a,b}$), 3.53 - 3.45 (m, 2H, H_8), 3.34 (s, 3H, H_{16}), 3.29 (s, 3H, H_{16}), 3.01 – 2.86 (m, 2H, D_4, D_5), 2.26 - 2.10 (m, 2H, D_{3eq}, D_{6eq}), 2.07 - 1.95 (m, 2H, D_{3ax}, D_{6ax}).

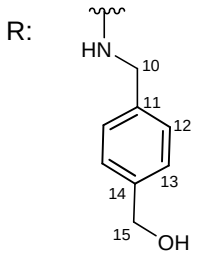
^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.4, 174.2 (C_9); 166.3, 166.0, 165,9, 165.8 (CO_{BZ}); 137.9, 137.5, 137.5, 137.4 (C_{11}, C_{14}); 133.9, 133.7, 133.5, 133.4 (CH_{BZ}); 130.1, 130.0, 129.9, 129.9 (CH_{BZ}); 129.2, 129.0 ($Cquat_{BZ}$); 128.7, 128.5, 128.4, 128.2, 128.2 (CH_{BZ}); 127.9, 127.9 (C_{12},

C_{13}); 97.2 (C_1); 75.3 (C_{D1}); 74.6, 74.5 (C_{15}); 74.5 (C_{D2}); 71.5 (C_2); 70.3 (C_3); 70.1 (C_5); 69.7 (C_7); 66.8 (C_4); 63.1 (C_6); 58.3, 58.2 (C_{16}); 52.3, 52.2 (C_{16}); 43.4 (C_8); 43.3 (C_{10}); 41.7, 41.0 (C_{D4}, C_{D5}); 28.8 (C_{D3}); 28.5 (C_{D6}).

2.4.5.7 N^1,N^2-bis(4-(hydroxymethylene)benzyl)amide, 2.10f

1,2-Cyclohexanedicarboxamides-N^1,N^2-bis(4-(hydroxymethylene)benzyl)-4-(2-chloroethoxy)-5-[(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)oxy]- (1*S*,2*S*,4*S*,5*S*)

Prepared according to general procedure 2, using amine **2.12f.**



2.12f

Yield: 86%

MS (ESI): calculated for $[C_{60}H_{59}ClN_2O_{15}Na]^+$: 1106.6; found: 1105.6

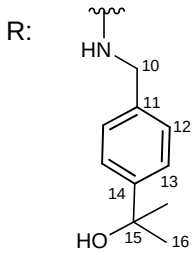
^{1}H NMR (400 MHz, CD_3OD): δ = 8.15 – 7.92 (m, 6H, H_{Bz}), 7.80 – 7.75 (m, 2H, H_{Bz}), 7.70 – 7.60 (m, 2H, H_{Bz}), 7.55 – 7.40 (m, 6H, H_{13}, H_{Bz}), 7.38 – 7.20 (m, 12H, H_{12}, H_{Bz}); 6.10 (t, 1H, H_4, $J_{4\text{-}3} = J_{4\text{-}5}$= 10.0 Hz), 6.00 (dd, 1H, H_3, $J_{3\text{-}4}$ = 10.0 Hz, $J_{3\text{-}2}$= 3.3), 5.86 (dd, 1H, H_2, $J_{2\text{-}1}$ = 1.7 Hz, $J_{2\text{-}3}$= 3.3 Hz), 5.39 (d, 1H, H_1, $J_{1\text{-}2}$= 1.7 Hz), 4.79 – 4.74 (m, 1H, H_{6a}), 4.66 – 4.53 (m, 6H, H_{15}, H_{6b}, H_5), 4.42 - 4.30 (m, 4H, $H_{10a,b}$), 4.23 – 4.18 (m, 1H, D_2), 3.92 – 3.87 (m, 1H, D_1), 3.83 - 3.76 (m, 1H, H_{7a}), 3.72 - 3.63 (m, 1H, H_{7a}), 3.60 - 3.57 (m, 2H, H_8), 3.17 – 2.98 (m, 2H, D_4, D_5), 2.13 - 1.99 (m, 2H, D_3, D_6).

^{13}C NMR (100 MHz, CD_3OD): δ = 177.4, 176.8 (C_9); 167.6, 167.2, 166.9, 166.9 (CO_{BZ}); 141.7, 139.2, 139.0 (C_{11}, C_{14}); 134.9, 134.8, 134.7, 134.6 (CH_{BZ}); 131.2 ($Cquat_{BZ}$); 130.9, 130.9 (CH_{BZ}); 130.7 ($Cquat_{BZ}$); 130.7 (CH_{BZ}); 130.4 ($Cquat_{BZ}$); 130.0, 129.9, 129.7, 129.6 (CH_{BZ}); 128.6, 128.5 (C_{12}, C_{13}); 98.2 (C_1); 76.5 (C_{D1}); 74.3 (C_{D2}); 72.2 (C_2); 72.0 (C_3); 71.0 (C_5); 70.8 (C_7); 68.5 (C_4); 65.1, 65.1 (C_{15}); 64.2 (C_6); 44.4 (C_8); 43.9 (C_{10}); 42.0, 42.0 (C_{D4}, C_{D5}); 30.1 (C_{D3}); 29.2 (C_{D6}).

2.4.5.8 N^1,N^2-bis(4-hydroxy(α,α-dimethyl)methylenebenzyl)amide, 2.10g

1,2-Cyclohexanedicarboxamides-N^1,N^2-bis(4-hydroxy(α,α-dimethyl)methylenebenzyl)-4-(2-chloroethoxy)-5-[(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)oxy]- (1*S*,2*S*,4*S*,5*S*)

Prepared according to general procedure 2, using amine **2.12g.**



2.10g

Yield: 95%

MS (ESI): calculated for $[C_{64}H_{67}ClN_2O_{15}Na]^+$: 1162.7; found: 1161.8

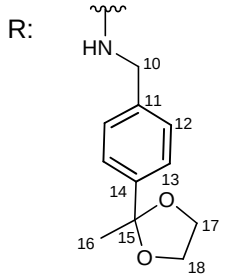
^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.06 – 7.92 (m, 6H, H_{Bz}), 7.74 – 7.66 (m, 2H, H_{Bz}), 7.60 – 7.46 (m, 3H, H_{Bz}), 7.41 – 7.31 (m, 11H, H_{Bz} ,H_{13}), 7.25 – 7.17 (m, 6H, H_{12}, H_{Bz}), 6.90 (t, 1H, H_{NH}, $J_{NH\text{-}10}$ = 5.7 Hz), 6.33 (t, 1H, H_{NH}, $J_{NH\text{-}10}$ = 5.7 Hz), 6.11 (t, 1H, H_4, $J_{4\text{-}3}$ = $J_{4\text{-}5}$= 10.0 Hz), 5.87 (dd, 1H, H_3, $J_{3\text{-}4}$ = 10.0 Hz, $J_{3\text{-}2}$= 3.1), 5.66 (dd, 1H, H_2, $J_{2\text{-}1}$ = 1.7 Hz, $J_{2\text{-}3}$= 3.1 Hz), 5.24 (d, 1H, H_1, $J_{1\text{-}2}$= 1.7 Hz), 4.70 – 4.63 (m, 1H, H_{6a}), 4.51 – 4.44 (m, 2H, H_{6b}, H_5), 4.42 - 4.32 (m, 2H, H_{10a}), 4.31 – 4.19 (m, 2H, H_{10a}), 4.08 – 4.04 (m, 1H, D_2), 3.78 – 3.73 (m, 1H, D_1), 3.77 - 3.55 (m, 2H, H_7), 3.52 - 3.47 (m, 2H, H_8), 3.03 – 2.89 (m, 2H, D_4, D_5), 2.28 – 1.92 (m, 4H, D_3, D_6), 1.51 (s, 3H, H_{16}), 1.50 (s, 3H, H_{16}), 1.46 (s, 3H, H_{16}), 1.43 (s, 3H, H_{16}).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.4, 174.3 (C_9); 166.3, 165.9, 165.9, 165.8 (CO_{BZ}); 148.5, 148.3 (C_{14}); 136.8, 136.8 (C_{11}); 133.9, 133.7, 133.5, 133.3 (CH_{BZ}); 130.1, 130.0, 129.9, 129.9 (CH_{BZ}); 129.2, 129.0 ($Cquat_{BZ}$); 128.8, 128.7, 128.5 (CH_{BZ}); 127.6, 127.6 (C_{12}); 124.9, 124.9 (C_{13}); 97.2 (C_1); 75.3 (C_{D1}); 74.5 (C_{D2}); 72.5, 72.5 (C_{15}); 71.5 (C_2); 70.3 (C_3); 70.1 (C_5); 69.7 (C_7); 66.8 (C_4); 63.1 (C_6); 58.3, 58.2 (C_{16}); 52.3, 52.2 (C_{16}); 43.3, 43.2 (C_{10} ,C_8); 41.8, 41.1 (C_{D4}, C_{D5}); 31.9, 31.9, 31.9, 31.8 (C_{16}); 28.8, 28.6 (C_{D3}, C_{D6}).

2.4.5.9 N^1,N^2-bis(4-(2-methyl-1,3-dioxolan-2-yl)benzyl)amide, 2.10h

1,2-Cyclohexanedicarboxamides-N^1,N^2-bis(4-(2-methyl-1,3-dioxolan-2-yl)benzyl)-4-(2-chloroethoxy)-5-[(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)oxy]- (1*S*,2*S*,4*S*,5*S*)

Prepared according to general procedure 2, using amine **2.12h.**



2.10h

Yield: 80%

MS (ESI): calculated for $[C_{66}H_{67}ClN_2O_{17}Na]^+$: 1218.7; found: 1218.8

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.06 – 8.02 (m, 2H, H_{Bz}), 8.01 – 7.96 (m, 4H, $H_{Bz,}$), 7.75 – 7.70 (m, 2H, H_{Bz}), 7.60 – 7.46 (m, 3H, H_{Bz}), 7.43–7.32 (m, 11H, H_{Bz}, H_{12}), 7.26 – 7.19 (m, 6H, H_{Bz}, H_{13}), 7.01 (t, 1H, H_{NH}, $J_{NH\text{-}10}$ = 5.8 Hz), 6.38 (t, 1H, H_{NH}, $J_{NH\text{-}10}$ = 5.8 Hz), 6.10 (t, 1H, H_4, $J_{4\text{-}3} = J_{4\text{-}5}$= 10.0 Hz), 5.89 (dd, 1H, H_3, $J_{3\text{-}4}$ = 10.0 Hz, $J_{3\text{-}2}$= 3.3), 5.67 (dd, 1H, H_2, $J_{2\text{-}1}$ = 1.7 Hz, $J_{2\text{-}3}$= 3.3 Hz), 5.25 (brs, 1H, H_1), 4.72 – 4.60 (m, 1H, H_{6a}), 4.53 – 4.21 (m, 6H, H_{6b}, $H_{10a,b}$, H_5), 4.09 - 4.04 (m, 1H, D_2), 4.03 - 3.96 (m, 4H, $H_{18a,\ 17a}$), 3.78 – 3.74 (m, 1H, D_1), 3.74 - 3.59 (m, 6H, $H_{7,}$ $H_{18b,\ 17b}$), 3.53 - 3.47 (m, 2H, H_8), 3.03 – 2.87 (m, 2H, D_4, D_5), 2.26 - 2.10 (m, 2H, D_{3eq}, D_{6eq}), 2.10 - 1.95 (m, 2H, D_{3ax}, D_{6ax}), 1.60 (s, 3H, H_{16}), 1.54 (s, 3H, H_{16}).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.3, 174.3 (C_9); 166.3, 166.0, 166.0, 165.9 (CO_{BZ}); 142.7, 142.6 (C_{11}); 138.1 (C_{14}); 133.9, 133.8, 133.6, 133.4 (CH_{BZ}); 130.1, 130.0, 129.9 (CH_{BZ}); 129.2, 129.0, 129.0 ($Cquat_{BZ}$); 128.8, 128.7, 128.7, 128.5 (CH_{BZ}); 127.7 (C_{13}); 125.8, 125.8 (C_{12}); 108,9 (C_{15}); 97.3 (C_1); 75.3 (C_{D1}); 74.6 (C_{D2}); 71.5 (C_2); 70.4 (C_3); 70.1 (C_5); 69.8 (C_7); 66.8 (C_4); 64.6, 64.6 (C_{16}, C_{17}); 63.9 (C_6); 43.3, 43.2 (C_8, C_{10}); 41.9, 41.7 (C_{D4}, C_{D5}); 28.8 (C_{D3}); 28.6 (C_{D6}); 27.8, 27.8 (C_{16});

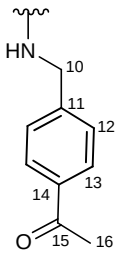
2.4.5.10 *N*^{1},*N*^{2}-bis(4-acetylbenzyl)amide, 2.10i

1,2-Cyclohexanedicarboxamides-*N*^{1},*N*^{2}-bis(4-acetylbenzyl)-4-(2-chloroethoxy)-5-[(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)oxy]- (1*S*,2*S*,4*S*,5*S*)

The acetal **2.10h** (60 mg, 0.05mmol, 1 eq) was dissolved in a mixture of acetone and water (10/1, 0.5ml) and to this solution pyridinium 4-toluenesulfonate (PPTS, 1.2 mg, 0.005 mmol, 0.1 eq) was added. The reaction was stirred at 50°C for 4 h. The solvents were removed under

reduced pressure and the crude was purified by flash chromatography (hexane: EtOAc = 3:7) to afford 50 mg of product.

R:



2.10i

Yield: 90%

MS (ESI): calculated for $[C_{62}H_{59}ClN_2O_{15}Na]^+$: 1129.6; found: 1129.6

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.04 (d, 2H, H_{Bz}, J = 8.3 Hz), 7.98 (d, 2H, H_{Bz}, J = 8.3 Hz), 7.93 (d, 2H, H_{Bz}, J = 8.3 Hz), 7.85 (d, 2H, H_{13}, $J_{13\text{-}12}$ = 8.1 Hz), 7.80 (d, 2H, H_{13}, $J_{13\text{-}12}$ = 8.1 Hz), 7.65 (d, 2H, H_{Bz}, J = 8.3 Hz), 7.60 – 7.45 (m, 3H, H_{Bz}), 7.41 – 7.28 (m, 11H, H_{12}, H_{Bz}), 7.22 – 7.16 (m, 2H, H_{Bz}), 7.06 (t, 1H, H_{NH}, $J_{NH\text{-}10}$ = 5.8 Hz), 6.36 (t, 1H, H_{NH}, $J_{NH\text{-}10}$ = 5.8 Hz), 6.12 (t, 1H, H_4, $J_{4\text{-}3}$ = $J_{4\text{-}5}$= 10.0 Hz), 5.86 (dd, 1H, H_3, $J_{3\text{-}4}$ = 10.0 Hz, $J_{3\text{-}2}$= 3.2), 5.66 (dd, 1H, H_2, $J_{2\text{-}1}$ = 1.7 Hz, $J_{2\text{-}3}$= 3.3 Hz), 5.24 (d, 1H, H_1, $J_{2\text{-}1}$ = 1.7 Hz), 4.73 – 4.63 (m, 1H, H_{6a}), 4.53 – 4.32 (m, 6H, H_{6b}, $H_{10a,b}$, H_5), 4.10 - 4.06 (m, 1H, D_2), 3.77 – 3.74 (m, 1H, D_1), 3.72 – 3.60 (m, 2H, H_7), 3.54 - 3.50 (m, 2H, H_8), 3.09 – 2.95 (m, 2H, D_4, D_5), 2.53 (s, 3H, H_{16}), 2.45 (s, 3H, H_{16}), 2.26 – 1.99 (m, 4H, D_3, D_6).

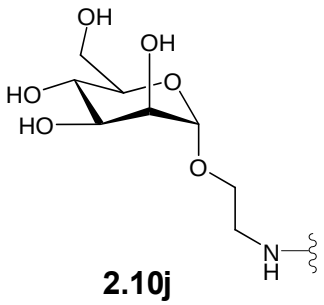
^{13}C NMR (100 MHz, $CDCl_3$): δ = 197.8, 197.8 (C_{15}); 174.6, 174.5 (C_9); 166.3, 166.1, 166.1, 165.7 (CO_{BZ}); 144.0, 143.9 (C_{11}); 136.4, 136.3 (C_{14}); 134.0, 133.8, 133.7, 133.4 (CH_{BZ}); 130.1, 130.0, 129.9, 129.9 (CH_{BZ}); 129.1, 128.9 ($Cquat_{BZ}$); 128.9, 128.9 (CH_{BZ}), 128.8 ($Cquat_{BZ}$); 128.7, 128.6 (CH_{BZ}, C_{13}); 127.7, 127,7 (C_{12}); 97.2 (C_1); 75.3 (C_{D1}); 74.5 (C_{D2}); 71.5 (C_2); 70.4 (C_3); 70.1 (C_5); 69.8 (C_7); 66.7 (C_4); 63.0 (C_6); 43.4 (C_8); 43.3, 43.2 (C_{10}); 41.7, 41.0 (C_{D4},C_{D5}); 29.1 (C_{D3}); 28.7 (C_{D6}); 26.8, 26.7 (C_{16}).

2.4.5.11 N^1,N^2-bis(2-(α-D-mannopyranosyloxy)ehyl)amide, 2.10j

1,2-Cyclohexanedicarboxamides-N^1,N^2-bis(2-(α-D-mannopyranosyloxy)ehyl)-4-(2-chloroethoxy)-5-[(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)oxy]- (1*S*,2*S*,4*S*,5*S*)

A solution of PFP scaffold **2.9** (45 mg, 0.0038 mmol 1 eq.) in H_2O/THF (1:2, 0.1 mL) was added to the solution of **2.12j** (51 mg, 0.23 mmol, 6 eq.) in H_2O/THF (1:2, 0.1 mL) over 8 hours at 35°C. The reaction was stirred for additional 16 h at 35°C. The solvent was evaporated under

reduced pressure. The crude was purified by flash chromatography (DCM with gradient of methanol from 0% to 20%, 10% H_2O in methanol) to afford 40 mg of pure product.



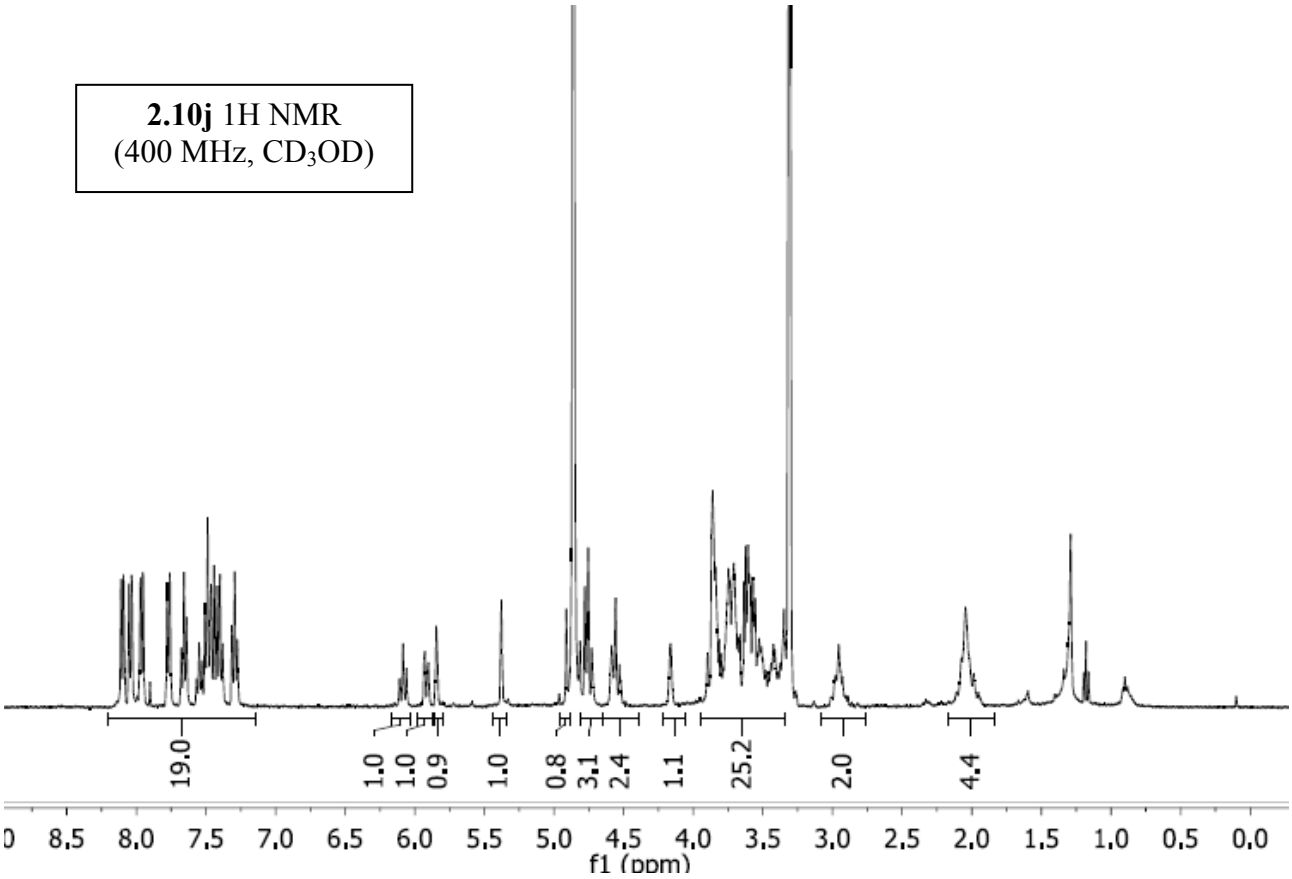
2.10j

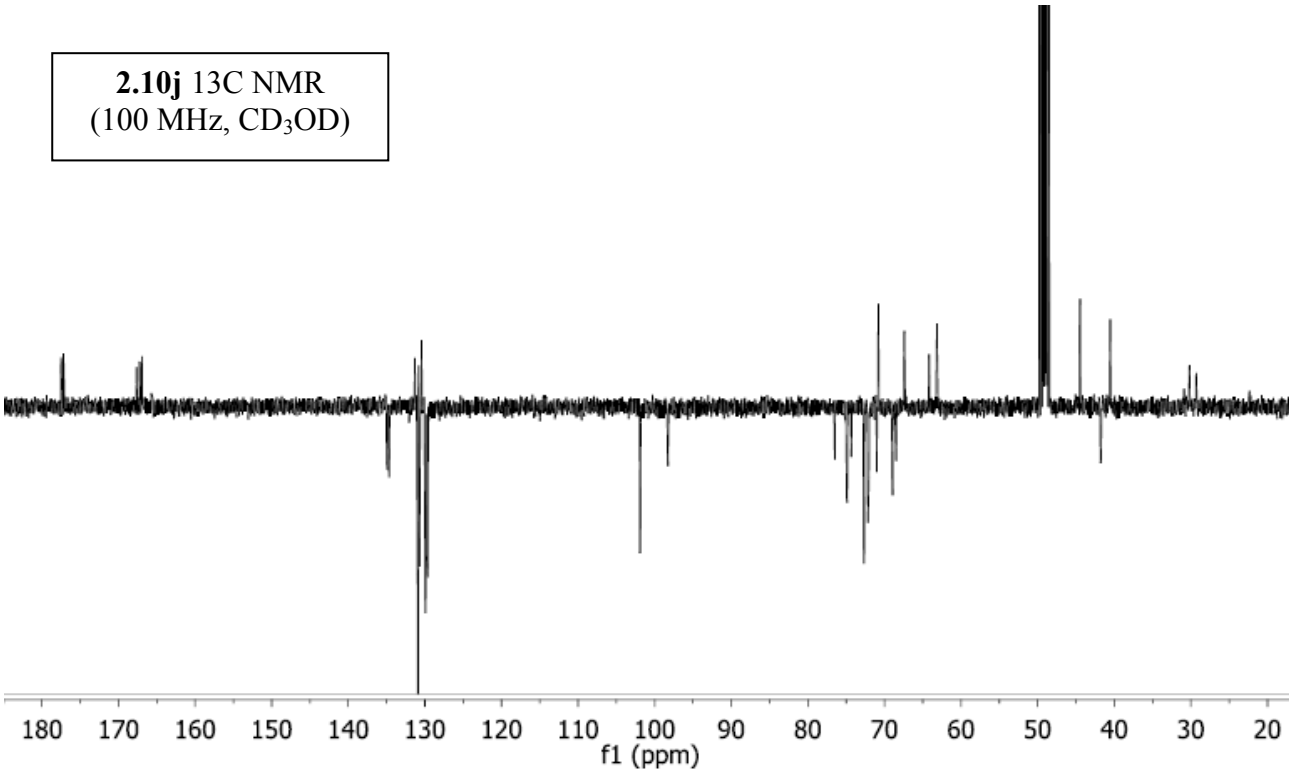
Yield = 83 %;

$[\alpha]_D^{20}$ = - 2.1 (c = 0.31 in ethanol)

MS (ESI) calculated for: $[C_{60}H_{71}ClN_2NaO_{25}]^+$: 1278.6; found: 1278.4

2.10j 1H NMR
(400 MHz, CD_3OD)

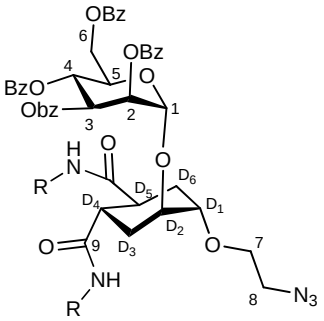




2.4.6 Synthesis and characterization of 1,2-Cyclohexanedicarboxamides-4-(2-azidoethoxy)-5-[(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)oxy]-(1*S*,2*S*,4*S*,5*S*), 2.11a-j

2.4.6.1 General procedure 3

To a solution of the chloroethyl derivative **2.10** (1 eq) in DMF (0.3 M) sodium azide (5 eq) and tetrabutylammonium iodide (0.1 eq) were added. The reaction was warmed at 45°C and stirred for 3 days. The solvent was removed at reduced pressure and the crude residue was purified by flash chromatography (silica, hexane with gradient of EA from 30% to 50%) to afford the pure product.

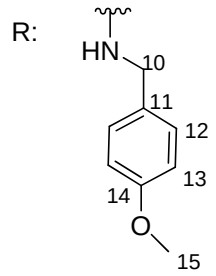


*General structure and numbering of pseudobimannoside **2.11a-j** bis-amides in the NMR characterizations*

2.4.6.2 N^1,N^2-bis(4-methoxybenzyl)amide, 2.11a

1,2-Cyclohexanedicarboxamides-N^1,N^2-bis(4-methoxybenzyl)-4-(2-azidoethoxy)-5-[(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)oxy]- (1*S*,2*S*,4*S*,5*S*)

Prepared according to general procedure 3, starting from chloride **2.10a.**



2.11a

Yield: 86%.

$[\alpha]^{20}_D$ = - 29.9 (c: 0.25, $CHCl_3$).

MS (FAB): calculated for $[C_{60}H_{60}N_5O_{15}]^+$: 1090; found: 1090

calculated for $[C_{60}H_{59}N_5O_{15}Na]^+$: 1112; found: 1112

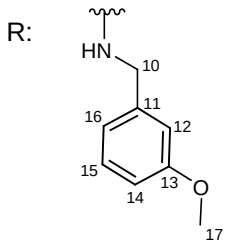
^{1}H NMR (400 MHz, $CDCl_3$): 8.07 (dd, 2H, H_{BZ}, $J_{o\text{-}p}$ = 1.2 Hz, $J_{o\text{-}m}$ = 8.4 Hz), 8.02 (dd, 2H, H_{BZ}, $J_{o\text{-}p}$ = 1.2 Hz, $J_{o\text{-}m}$ = 8.4 Hz),7.97 (dd, 2H, H_{BZ}, $J_{o\text{-}p}$ = 1.2 Hz, $J_{o\text{-}m}$ = 8.4 Hz), 7.72 (dd, 2H, H_{BZ}, $J_{o\text{-}p}$ = 1.2 Hz, $J_{o\text{-}m}$ = 8.4 Hz), 7.64-7.49 (m, 3H, H_{BZ}), 7.47-7.35 (m, 7H, H_{BZ}), 7.26-7.16 (m, 6H, H_{BZ}, H_{12}), 6.88-6.79 (m, 4H, H_{13}), 6.74 (t, 1H, NH, $J_{NH\text{-}10}$ = 5.2 Hz), 6.17 (t, 1H, NH, $J_{NH\text{-}10}$ = 5.2 Hz), 6.13 (dd, 1H, H_4, $J_{4\text{-}5}$ = 10.4 Hz, $J_{4\text{-}3}$ = 10 Hz), 5.88 (dd, 1H, H_3, $J_{3\text{-}2}$ = 3.2 Hz, $J_{3\text{-}4}$ = 10.4 Hz), 5.67 (dd, 1H, H_2, $J_{2\text{-}1}$ = 1.6 Hz, $J_{2\text{-}3}$ = 3.2 Hz), 5.27 (d, 1H, H_1, $J_{1\text{-}2}$ = 1.6 Hz), 4.73-4.65 (m, 1H, H_{6b}), 4.55-4.46 (m, 2H, H_5, H_{6a}), 4.42-4.33 (m, 2H, H_{10a}), 4.30-4.19 (m, 2H, H_{10b}), 4.08 (m, 1H, D_2), 3.78 (s, 3H, H_{15}), 3.77 (m, 1H, D_1), 3.73 (s, 3H, H_{15}), 3.64-3.54 (m, 2H, $H_{7a,b}$), 3.34-3.21 (m, 2H, $H_{8a,b}$) 3.00-2.87 (m, 2H, D_4, D_5), 2.29-2.14 (m, 2H, D_{3eq}, D_{6eq}), 2.09-1.98 (m, 2H, D_{3ax}, D_{6ax}).

^{13}C NMR (100 MHz, $CDCl_3$): 174.1, 174.0 (**C**O); 166.2, 165.9, 165.8, 165.7 (**C**O_{BZ}); 159.0, 159.0 (C_{14}); 133.8, 133.7, 133.4, 133.3 (**C**H_{BZ}); 130.6, 130.4, 129.9, 129.0, 128.9 (**C**$_{quatBZ}$, C_{11}); 130.1, 130.0, 129.9, 129.2, 129.1, 128.8, 128.6, 128.6, 128.4 (**C**H_{BZ}, C_{12}); 114.2, 114.1 (C_{13}); 97.1 (C_1); 75.5 (C_{D1}); 74.4 (C_{D2}); 71.5 (C_2); 70.2 (C_3); 70.0 (C_5); 68.6 (C_7); 66.8 (C_4); 63.0 (C_6); 55.4, 55.3 (C_{15}); 50.8 (C_8) 43.2, 43.0 (C_{10}); 41.8, 41.0 (C_{D4}, C_{D5}); 28.7, 28.4 (C_{D3}, C_{D6}).

2.4.6.3 N^1,N^2-bis(3-methoxybenzyl)amide, 2.11b

1,2-Cyclohexanedicarboxamides-N^1,N^2-bis(3-methoxybenzyl)-4-(2-azidoethoxy)-5-[(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)oxy]- (1*S*,2*S*,4*S*,5*S*)

Prepared according to general procedure 3, starting from chloride **2.10b.**



2.10b

Yield: 99%

MS (FAB): calculated for $[C_{60}H_{59}N_5O_{15}]^+$: 1090; found: 1090

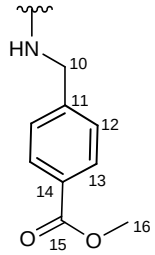
calculated for $[C_{60}H_{59}N_5O_{15}Na]^+$: 1112; found: 1112

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.06 – 7.90 (m, 6H, H_{Bz}), 7.71 – 7.65 (m, 2H, H_{Bz}), 7.60 – 7.46 (m, 3H, H_{Bz}), 7.43–7.32 (m, 7H, H_{Bz}), 7.25 – 7.13 (m, 4H, H_{Bz}, H_{15}), 6.88 - 6.67 (m, 7H, H_{12}, H_{14}, H_{16}, H_{NH}), 6.25 – 6.16 (brs, 1H, H_{NH}), 6.11 (t, 1H, H_4, $J_{4\text{-}3} = J_{4\text{-}5}$= 10.0 Hz), 5.86 (dd, 1H, H_3, $J_{3\text{-}4}$ = 10.0 Hz, $J_{3\text{-}2}$= 3.3), 5.65 (brs, 1H, H_2), 5.24 (brs, 1H, H_1), 4.70 – 4.62 (m, 1H, H_{6a}), 4.55 – 4.30 (m, 4H, H_{6b}, H_{10a}, H_5), 4.30-4.19 (m, 2H, H_{10b}), 4.06 (brs, 1H, D_2), 3.86 – 3.65 (m, 7H, H_{17}, H_{7a}), 3.67 – 3.55 (m, 3H, D_1, H_{7b}), 3.36 - 3.17 (m, 2H, H_8), 3.01 – 2.87 (m, 2H, D_4, D_5), 2.26 - 2.11 (m, 2H, D_{3eq}, D_{6eq}), 2.10 - 1.95 (m, 2H, D_{3ax}, D_{6ax}),

2.4.6.4 N^1,N^2-bis(4-carbomethoxy)amide, 2.11c

1,2-Cyclohexanedicarboxamides-N^1,N^2-bis(4-carbomethoxy)-4-(2-azidoethoxy)-5-[(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)oxy]- (1*S*,2*S*,4*S*,5*S*)

Prepared according to general procedure 3, starting from chloride **2.10c.**

R: 

2.11c

Yield: 78%

MS (ESI): calculated for $[C_{62}H_{59}N_5O_{17}Na]^+$: 1168.1; found: 1168.5

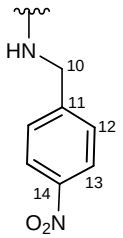
^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.06 – 8.03 (m, 2H, H_{Bz}), 7.99 – 7.86 (m, 8H, H_{Bz}, H_{13}), 7.72 – 7.64 (m, 2H, H_{Bz}), 7.59 – 7.51 (m, 2H, H_{Bz}), 7.50–7.45 (m, 1H, H_{Bz}), 7.41 – 7.17 (m, 13H, H_{Bz}, H_{12}), 7.01 (t, 1H, H_{NH}, $J_{NH\text{-}10}$ = 5.62 Hz), 6.34 (t, 1H, H_{NH}, $J_{NH\text{-}10}$ = 5.8 Hz), 6.12 (t, 1H, H_4, $J_{4\text{-}3}$ = $J_{4\text{-}5}$= 10.0 Hz), 5.87 (dd, 1H, H_3, $J_{3\text{-}4}$ = 10.0 Hz, $J_{3\text{-}2}$= 3.3), 5.66 (dd, 1H, H_2, $J_{2\text{-}1}$ = 1.7 Hz, $J_{2\text{-}3}$= 3.3 Hz), 5.24 (d, 1H, H_1, $J_{1\text{-}2}$= 1.7 Hz), 4.74 – 4.62 (m, 1H, H_{6a}), 4.51 – 4.30 (m, 6H, H_{6b}, $H_{10a,b}$, H_5), 4.12 - 4.04 (m, 1H, D_2), 4.87 (s, 3H, H_{16}), 3.84 (s, 3H, H_{16}), 3.79 – 3.74 (m, 1H, D_1), 3.71 - 3.51 (m, 2H, $H_{7a,b}$), 3.40 - 3.16 (m, 2H, H_8), 3.16 – 2.89 (m, 2H, D_4, D_5), 2.28 - 2.11 (m, 2H, D_{3eq}, D_{6eq}), 2.11 - 1.98 (m, 2H, D_{3ax}, D_{6ax}),

^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.5, 174.5 (C_9); 167.0, 167.0 (C_{15}); 166.3, 166.1, 166.0, 165.7 (CO_{BZ}); 143.8, 143.7 (C_{11}); 133.9, 133.7, 133.6, 133.4 (CH_{BZ}); 130.1, 130.1, 130.0, 129.9, 129.9 (CH_{BZ}); 129.3, 129.3, 129.1, 128.9, 128.9 ($Cquat_{BZ}$, C_{14}); 128.8, 128.6, 128.5 (CH_{BZ}); 127.6, 127.5 (C_{12}, C_{13}); 97.2 (C_1); 75.6 (C_{D1}); 74.2 (C_{D2}); 73.1 (C_2); 71.5 (C_3); 70.1 (C_5); 68.7 (C_7); 66.7 (C_4); 63.0 (C_6); 52.3, 52.2 (C_{16}); 50.9 (C_8); 43.3, 43.2, (C_{10}); 41.7, 41.0 (C_{D4}, C_{D5}); 28.9, 28.8 (C_{D3} , C_{D6}).

2.4.6.5 N^1,N^2-bis(4-nitrobenzyl)amide, 2.11d

1,2-Cyclohexanedicarboxamides-N^1,N^2-bis(4-nitrobenzyl)-4-(2-azidoethoxy)-5-[(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)oxy]- (1*S*,2*S*,4*S*,5*S*)

Prepared according to general procedure 3, starting from chloride **2.10d.**

R: 

2.11d

Yield: 65%

MS (FAB): calculated for $[C_{58}H_{53}N_7O_{17}]^+$: 1120; found: 1120

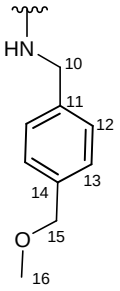
calculated for $[C_{58}H_{53}N_7O_{17}Na]^+$: 1143; found: 1142

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.14 – 7.90 (m, 10H, H_{Bz}, H_{13}), 7.68 – 7.64 (m, 2H, H_{Bz}), 7.61 – 7.47 (m, 3H, H_{Bz}), 7.45 – 7.33 (m, 10H, H_{13}, H_{Bz}), 7.26–7.11 (m, 3H, H_{NH}, H_{Bz}), 6.36 (brs, 1H H_{NH}), 6.14 (t, 1H, H_4, $J_{4\text{-}3} = J_{4\text{-}5}$= 10.0 Hz), 5.84 (dd, 1H, H_3, $J_{3\text{-}4}$ = 10.0 Hz, $J_{3\text{-}2}$= 3.3), 5.68 (dd, 1H, H_2, $J_{2\text{-}1}$ = 1.7 Hz, $J_{2\text{-}3}$= 3.3 Hz), 5.24 (d, 1H, H_1, $J_{1\text{-}2}$= 1.7 Hz), 4.77 – 4.64 (m, 1H, H_{6a}), 4.62– 4.35 (m, 6H, H_{6b}, $H_{10a,b}$, H_5), 4.10 - 4.04(brs, D_2), 3.79 – 3.74 (m, 1H, D_1), 3.71 - 3.51 (m, 2H, $H_{7a,b}$), 3.40 - 3.16 (m, 2H, H_8), 3.15 – 2.90 (m, 2H, D_4, D_5), 2.28 - 2.10 (m, 2H, D_{3eq}, D_{6eq}), 2.11 – 2.20 (m, 2H, D_{3ax}, D_{6ax}).

2.4.6.6 *N*^{1},*N*^{2}-bis(4-(methoxymethyl)benzyl)amide, 2.11e

1,2-Cyclohexanedicarboxamides-*N*^{1},*N*^{2}-bis(4-methoxybenzyl)-4-(2-azidoethoxy)-5-[(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)oxy]- (1*S*,2*S*,4*S*,5*S*)

Prepared according to general procedure 3, starting from chloride **2.10e.**

R: 

2.11e

Yield: 66%

MS (ESI): calculated for $[C_{62}H_{63}N_5O_{15}Na]^+$: 1141.2; found: 1140.6

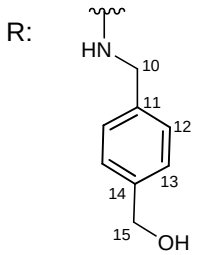
^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.06 – 7.92 (m, 6H, H_{Bz}), 7.72 – 7.68 (m, 2H, H_{Bz}), 7.60 – 7.47 (m, 3H, H_{Bz}), 7.42 – 7.32 (m, 7H, H_{13}, H_{Bz}), 7.29 – 7.19 (m, 10H, H_{13}, H_{Bz}), 6.79 (t, 1H, H_{NH}, $J_{NH\text{-}10}$ = 5.7 Hz), 6.17 (t, 1H, H_{NH}, $J_{NH\text{-}10}$ = 5.7 Hz), 6.11 (t, 1H, H_4, $J_{4\text{-}3}$ = $J_{4\text{-}5}$= 10.0 Hz), 5.86 (dd, 1H, H_3, $J_{3\text{-}4}$ = 10.0 Hz, $J_{3\text{-}2}$= 3.3), 5.64 (dd, 1H, H_2, $J_{2\text{-}1}$ = 1.7 Hz, $J_{2\text{-}3}$= 3.3 Hz), 5.24 (d, 1H, H_1, $J_{1\text{-}2}$= 1.7 Hz), 4.70 – 4.63 (m, 1H, H_{6a}), 4.51 – 4.20 (m, 10H, H_{15}, H_{6b}, $H_{10a,b}$, H_5), 4.08 - 4.04 (m, 1H, D_2), 3.78 – 3.74 (m, 1H, D_1), 3.62 - 3.50 (m, 2H, $H_{7a,b}$), 3.34 (s, 3H, H_{16}), 3.32 - 3.17 (m, 2H, H_8), 3.29 (s, 3H, H_{16}), 3.01 – 2.86 (m, 2H, D_4, D_5), 2.26 - 2.10 (m, 2H, D_{3eq}, D_{6eq}), 2.07 - 1.95 (m, 2H, D_{3ax}, D_{6ax}),

^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.2, 174.2 (C_9); 166.3, 165.9, 165.9, 165.8 (CO_{BZ}); 138.0, 137.9, 137.5, 137.4 (C_{11}, C_{14}); 133.9, 133.7, 133.5, 133.4 (CH_{BZ}); 130.2, 130.0, 130.0, 129.9 (CH_{BZ}); 129.2, 129.0 ($Cquat_{BZ}$); 128.8, 128.7, 128.7, 128.5, 128.2, 128.2, (CH_{BZ}); 127.9, 127.9 (C_{12}, C_{13}); 97.3 (C_1); 75.6 (C_{D1}); 74.6, 74.5 (C_{15}); 74.4 (C_{D2}); 71.5 (C_2); 70.3 (C_3); 70.1 (C_5); 68.7 (C_7); 66.8 (C_4); 63.1 (C_6); 58.3, 58.2 (C_{16}); 52.3, 52.2 (C_{16}); 50.9 (C_8); 43.4, 43.4 (C_{10}); 41.8, 41.1 (C_{D4}, C_{D5}); 28.8, 28.6 (C_{D6}, C_{D3}).

2.4.6.7 N^1,N^2-bis(4-(hydroxymethylene)benzyl)amide, 2.11f

1,2-Cyclohexanedicarboxamides-N^1,N^2-bis(4-(hydroxymethylene)benzyl)-4-(2-azidoethoxy)-5-[(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)oxy]- (1*S*,2*S*,4*S*,5*S*)

Prepared according to general procedure 3, starting from chloride **2.10f.**



2.11f

Yield: 98%

MS (ESI): calculated for $[C_{60}H_{59}N_5O_{15}Na]^+$: 1113.1; found: 1112.5

^{1}H NMR (400 MHz, CD_3OD): δ = 8.15 – 7.89 (m, 6H, H_{Bz}), 7.77 – 7.71 (m, 2H, H_{Bz}), 7.68 – 7.60 (m, 2H, H_{Bz}), 7.55 – 7.40 (m, 6H, H_{13}, H_{Bz}), 7.38 – 7.20 (m, 12H, H_{12}, H_{Bz}); 6.09 (t, 1H, H_4, $J_{4\text{-}3}$ = $J_{4\text{-}5}$= 10.0 Hz), 6.00 (dd, 1H, H_3, $J_{3\text{-}4}$ = 10.0 Hz, $J_{3\text{-}2}$= 3.3), 5.85 (dd, 1H, H_2, $J_{2\text{-}1}$ = 1.7 Hz, $J_{2\text{-}3}$= 3.3 Hz), 5.38 (brs, 1H, H_1), 4.79 – 4.74 (m, 1H, H_{6a}), 4.66 – 4.51 (m, 6H, H_{15}, H_{6b}, H_5),

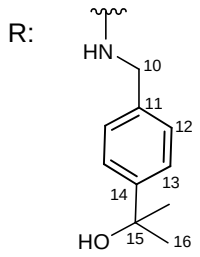
4.42 - 4.28 (m, 4H, $H_{10a,b}$), 4.23 – 4.16 (m, 1H, D_2), 3.92 – 3.87 (m, 1H, D_1), 3.80 - 3.53 (m, 2H, $H_{7a,b}$), 3.40 - 3.23 (m, 2H, H_8), 3.17 – 2.94 (m, 2H, D_4, D_5), 2.22 - 1.99 (m, 2H, D_3, D_6).

^{13}C NMR (100 MHz, CD_3OD): δ = 177.1, 176.8 (C_9); 167.6, 167.2, 166.9, 166.9 (CO_{BZ}); 139.2, 139.2, 139.1 (C_{11}, C_{14}); 134.9, 134.9, 134.7, 134.6 (CH_{BZ}); 131.3 ($Cquat_{BZ}$); 130.9, 130.9, 130.9 (CH_{BZ}); 130.8 ($Cquat_{BZ}$); 130.7 (CH_{BZ}); 130.4, 130.4 ($Cquat_{BZ}$); 130.0, 129.9, 129.7, 129.6 (CH_{BZ}); 128.6, 128.5, 128.3 (C_{12}, C_{13}); 98.1 (C_1); 76.6 (C_{D1}); 74.2 (C_{D2}); 72.2 (C_2); 72.0 (C_3); 71.0 (C_5); 70.8 (C_7); 68.5 (C_4); 65.1, 65.1 (C_{15}); 64.2 (C_6); 52.1 (C_8); 43.9 (C_{10}); 42.0, 41.9 (C_{D4}, C_{D5}); 29.9, 29.1 (C_{D3} ,C_{D6})

2.4.6.8 *N*^{1},*N*^{2}-bis(4-(hydroxyl(α,α-dimethyl)methylene)benzyl)amide, 2.11g

1,2-Cyclohexanedicarboxamides-*N*^{1},*N*^{2}-bis(4-(hydroxyl(α,α-dimethyl)methylene)benzyl)-4-(2-azidoethoxy)-5-[(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)oxy]- (1*S*,2*S*,4*S*,5*S*)

Prepared according to general procedure 3, starting from chloride **2.10g.**



2.11g

Yield: 82%

MS (ESI): calculated for $[C_{64}H_{67}N_5O_{15}Na]^+$: 1169.2; found: 1168.9

^{1}H NMR (400 MHz, CD_3OD): δ = 8.12 – 7.89 (m, 6H, H_{Bz}), 7.78 – 7.70 (m, 2H, H_{Bz}), 7.68 – 7.59 (m, 2H, H_{Bz}), 7.54 – 7.36 (m, 10H, H_{Bz} ,H_{13}), 7.36 – 7.30 (m, 2H, H_{Bz}), 7.30 – 7.17 (m, 6H, H_{12}, H_{Bz}), 6.08 (t, 1H, H_4, $J_{4-3} = J_{4-5}$= 10.0 Hz), 5.97 (dd, 1H, H_3, J_{3-4} = 10.0 Hz, J_{3-2}= 3.1), 5.83 (dd, 1H, H_2, J_{2-1} = 1.7 Hz, J_{2-3}= 3.1 Hz), 5.36 (d, 1H, H_1, J_{1-2}= 1.7 Hz), 4.74 – 4.70 (m, 1H, H_{6a}), 4.65 – 4.48 (m, 2H, H_{6b}, H_5), 4.35 - 4.26 (m, 2H, H_{10a}), 4.37 - 4.26 (m, 4H, $H_{10a,b}$), 4.21 – 4.16 (m, 1H, D_2), 3.91 – 3.84 (m, 1H, D_1), 3.75 - 3.67 (m, 1H, H_{7a}), 3.61 - 3.52 (m, 1H, H_{7b}), 3.38 - 3.23 (m, 2H, H_8), 3.15 – 2.94 (m, 2H, D_4, D_5), 2.22 - 1.99 (m, 2H, D_3, D_6), 1.48, 1.45 (s, 12H, H_{16}).

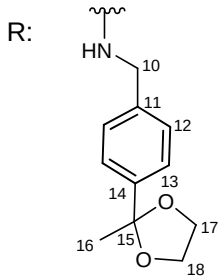
^{13}C NMR (100 MHz, CD_3OD): δ = 177.0, 176.7 (C_9); 167.6, 167.2, 166.9, 166.9 (CO_{BZ}); 149.9 (C_{14}); 138.2, 138.1 (C_{11}); 134.9, 134.9, 134.7, 134.6 (CH_{BZ}); 131.3 ($Cquat_{BZ}$); 130.9, 130.9 (CH_{BZ}); 130.8 ($Cquat_{BZ}$); 130.7 (CH_{BZ}); 130.4 ($Cquat_{BZ}$); 130.0, 129.9, 129.7, 129.6 (CH_{BZ});

128.3, 128.53 (C_{12}, C_{13}); 98.2 (C_1); 76.5 (C_{D1}); 74.4 (C_{D2}); 73.0 (C_2); 72.2 (C_2); 72.0 (C_3); 71.0 (C_5); 69.3 (C_7); 68.5 (C_4); 64.2 (C_6); 52.1 (C_8); 43.8 (C_{10}); 42.1, 42.0 (C_{D4}, C_{D5}); 32.1, 32.1, 32.1 (C_{16}), 29.8, 29.1 (C_{D3} ,C_{D6})

2.4.6.9 N^1,N^2-bis(4-(2-methyl-1,3-dioxolan-2-yl)benzyl)amide, 2.11h

1,2-Cyclohexanedicarboxamides-N^1,N^2-bis(4-(2-methyl-1,3-dioxolan-2-yl)-4-(2-azidoethoxy)-5-[(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)oxy]- (1*S*,2*S*,4*S*,5*S*)

Prepared according to general procedure 3, starting from chloride **2.10h.**



2.11h

Yield: 62%

MS (ESI) calculated for $[C_{66}H_{67}N_5O_{17}Na]^+$: 1225.2; found: 1224.9.

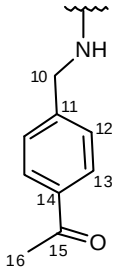
1H NMR (400 MHz, CDCl3): δ = 8.06 – 8.02 (m, 2H, H_{Bz}), 8.01 – 7.96 (m, 4H, $H_{Bz,}$), 7.75 – 7.70 (m, 2H, H_{Bz}), 7.60 – 7.46 (m, 3H, H_{Bz}), 7.43–7.32 (m, 11H, H_{Bz}, H_{12}), 7.26 – 7.19 (m, 6H, H_{Bz}, H_{13}), 6.81 (t, 1H, H_{NH}, $J_{NH\text{-}10}$ = 5.8 Hz), 6.21 (t, 1H, H_{NH}, $J_{NH\text{-}10}$ = 5.8 Hz), 6.10 (t, 1H, H_4, $J_{4\text{-}3}$ = $J_{4\text{-}5}$= 10.0 Hz), 5.86 (dd, 1H, H_3, $J_{3\text{-}4}$ = 10.0 Hz, $J_{3\text{-}2}$= 3.3), 5.66 (dd, 1H, H_2, $J_{2\text{-}1}$ = 1.7 Hz, $J_{2\text{-}3}$= 3.3 Hz), 5.24 (d, 1H, H_1, $J_{1\text{-}2}$= 1.7 Hz), 4.70 – 4.64 (m, 1H, H_{6a}), 4.53 – 4.20 (m, 6H, H_{6b}, $H_{10a,b}$, H_5), 4.09 - 4.04 (m, 1H, D_2), 4.03 - 3.91 (m, 4H, $H_{18a,\ 17a}$), 3.78 – 3.74 (m, 1H, D_1), 3.74 - 3.63 (m, 4H, $H_{18b,\ 17b}$), 3.61 – 3.52 (m, 2H, H_7), 3.34 - 3.17 (m, 2H, H_8), 3.03 – 2.87 (m, 2H, D_4, D_5), 2.30 - 2.10 (m, 2H, D_{3eq}, D_{6eq}), 2.10 - 2.95 (m, 2H, D_{3ax}, D_{6ax}), 1.60 (s, 3H, H_{16}), 1.55 (s, 3H, H_{16}).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.2, 172.2 (C_9); 166.3, 165.9, 165.9, 165.8 (CO_{BZ}); 142.7, 142.6 (C_{11}); 138.1 (C_{14}); 133.9, 133.8, 133.6, 133.4 (CH_{BZ}); 130.1, 130.0, 129.9 (CH_{BZ}); 129.2, 129.0, 129.0 ($Cquat_{BZ}$); 128.8, 128.7, 128.7, 128.5 (CH_{BZ}); 127.7 (C_{13}); 125.8, 125.8 (C_{12}); 108,9 (C_{15}); 97.3 (C_1); 75.5 (C_{D1}); 74.2 (C_{D2}); 71.5 (C_2); 70.3 (C_3); 70.1 (C_5); 68.7 (C_7); 66.8 (C_4); 64.6, 64.6 (C_{16}, C_{17}); 63.1 (C_6); 50.9 (C_8); 43.3 (C_{10}); 41.8, 41.1 (C_{D4}, C_{D5}); 28.8 (C_{D3}); 28.6 (C_{D6}); 27.8, 27.8 (C_{16});

2.4.6.10 N^1,N^2-bis(4-acetobenzyl)amide, 2.11i

1,2-Cyclohexanedicarboxamides-N^1,N^2-bis(4-acetylbenzyl)-4-(2-azidoethoxy)-5-[(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)oxy]- (1*S*,2*S*,4*S*,5*S*)

Prepared according to general procedure 3, starting from chloride **2.10i.**



2.11i

Yield: 98%

MS (ESI) calculated for $[C_{62}H_{59}N_5O_{15}Na]^+$: 1137.1; found: 1136.3.

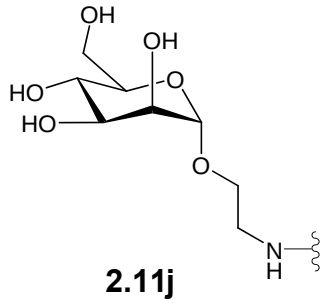
1H NMR (400 MHz, CDCl3): δ = 8.04 (d, 2H, H_{Bz}, J = 8.3 Hz), 7.98 (d, 2H, H_{Bz}, J = 8.3 Hz), 7.93 (d, 2H, H_{Bz}, J = 8.3 Hz), 7.85 (d, 2H, H_{13}, $J_{13\text{-}12}$ = 8.1 Hz), 7.80 (d, 2H, H_{13}, $J_{13\text{-}12}$ = 8.1 Hz), 7.65 (d, 2H, H_{Bz}, J = 8.3 Hz), 7.90 – 7.45 (m, 3H, H_{Bz}), 7.41 – 7.28 (m, 11H, H_{12}, H_{Bz}), 7.22 – 7.16 (m, 2H, H_{Bz}), 7.00 (t, 1H, H_{NH}, $J_{NH\text{-}10}$ = 5.8 Hz), 6.32 (t, 1H, H_{NH}, $J_{NH\text{-}10}$ = 5.8 Hz), 6.12 (t, 1H, H_4, $J_{4\text{-}3}$ = $J_{4\text{-}5}$= 10.0 Hz), 5.86 (dd, 1H, H_3, $J_{3\text{-}4}$ = 10.0 Hz, $J_{3\text{-}2}$= 3.2), 5.66 (m, 1H, H_2), 5.24 (br s, 1H, H_1), 4.73 – 4.63 (m, 1H, H_{6a}), 4.53 – 4.32 (m, 6H, H_{6b}, $H_{10a,b}$, H_5), 4.13 - 4.06 (m, 1H, D_2), 3.79 – 3.74 (m, 1H, D_1), 3.66 – 3.54 (m, 2H, H_7), 3.34 - 3.17 (m, 2H, H_8), 3.09 – 2.88 (m, 2H, D_4, D_5), 2.53 (s, 3H, H_{16}), 2.45 (s, 3H, H_{16}), 2.26 - 2.03 (m, 4H, D_3, D_6).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 197.8, 197.8 (C_{15}); 174.5, 174.5 (C_9); 166.3, 166.1, 166.1, 165.7 (CO_{BZ}); 144.0, 144.9 (C_{11}); 136.4, 136.3 (C_{14}); 134.0, 133.8, 133.6, 133.4 (CH_{BZ}); 130.1, 130.0, 129.9, 129.9 (CH_{BZ}); 129.1, 128.9, 129.0 ($Cquat_{BZ}$); 128.9, 128.9, 128.7, 128.5 (CH_{BZ}, C_{13}); 127.7, 127,6 (C_{12}); 97.2 (C_1); 75.3 (C_{D1}); 74.3 (C_{D2}); 71.5 (C_2); 70.4 (C_3); 70.2 (C_5); 68.8 (C_7); 66.7 (C_4); 63.0 (C_6); 50.9 (C_8); 43.3, 43.2 (C_{10}); 41.7, 41.0 (C_{D4},C_{D5}); 29.0 (C_{D3}); 28.9 (C_{D6}); 26.8, 26.7 (C_{16}).

2.4.6.11 N^1,N^2-bis(2-(α-D-mannopyranosyloxy)ehyl)amide, 2.11j

1,2-Cyclohexanedicarboxamides-N^1,N^2-bis(2-(α-D-mannopyranosyloxy)ehyl)-4-(2-azidoethoxy)-5-[(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)oxy]- (1*S*,2*S*,4*S*,5*S*)

Prepared according to general procedure 3, starting from chloride **2.10i.**

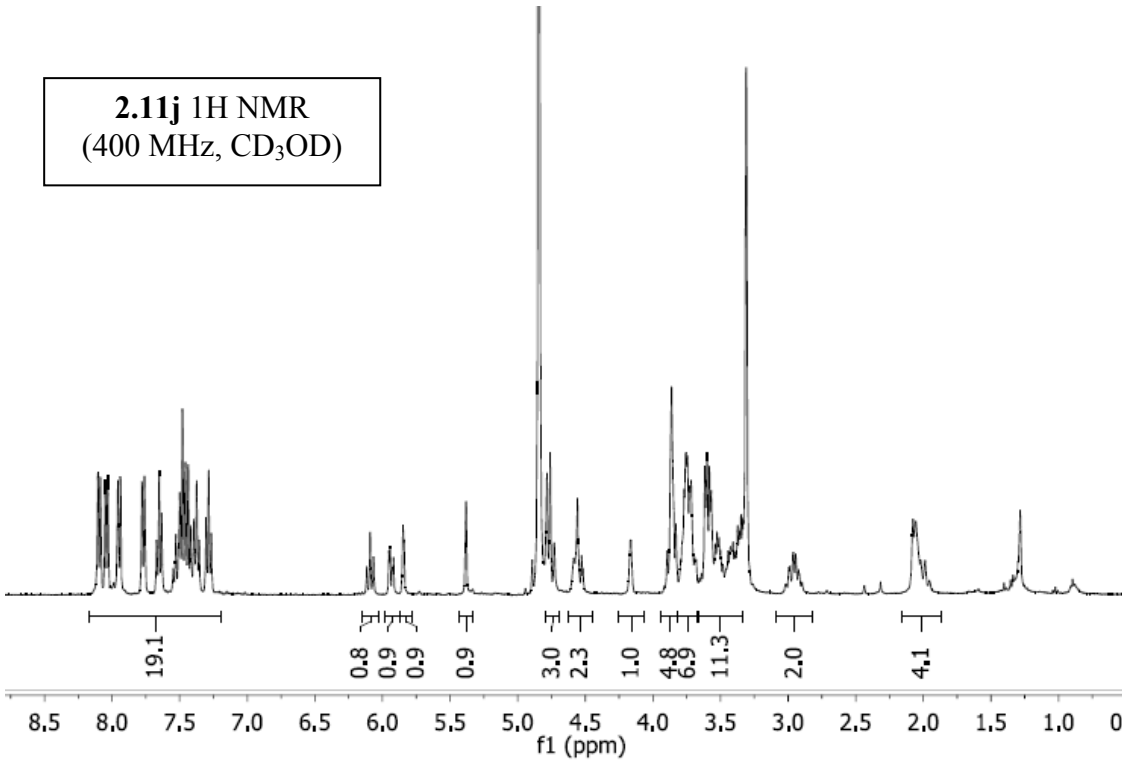


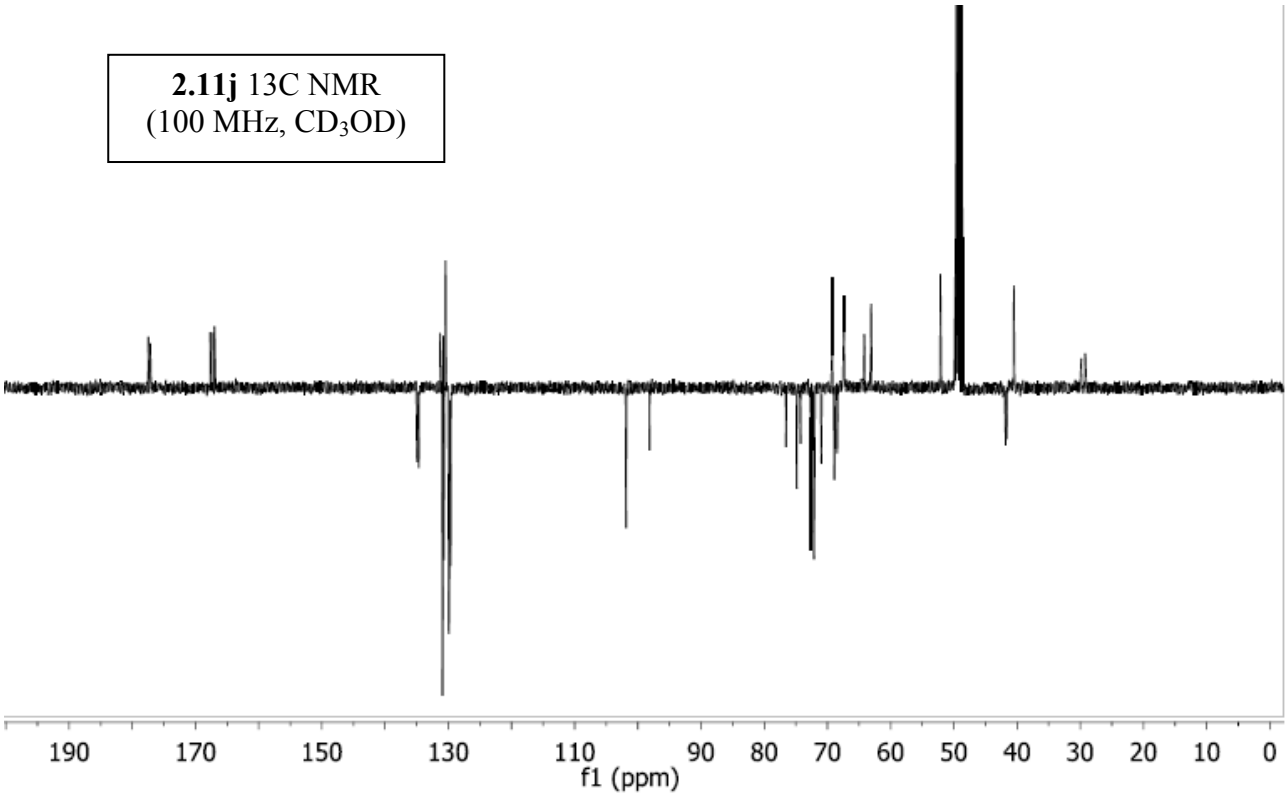
2.11j

Yield = 50 %;

$[\alpha]_D^{20}$ = + 14.5 (c = 0.22 in methanol)

MS (FAB): calculated for: $[C_{60}H_{71}N_5O_{25}Na]^+$: 1285; found: 1285

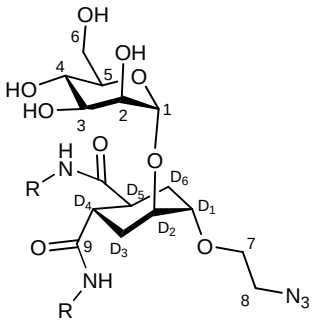




2.4.7 Synthesis and characterization of final DC-SIGN ligands 1,2-Cyclohexanedicarboxamide, 4-(2-azidoethoxy)-5-(α-D-mannopyranosyloxy),- (1*S*,2*S*,4*S*,5*S*), 2.2a-j

2.4.7.1 General procedure 4

Compound **2.11** was dissolved in dry methanol (c = 0.1 M), under nitrogen at room temperature, and 1M solution of sodium methoxide in MeOH (2 eq) was added. After reaction completion (1 h; TLC, DCM:MeOH = 9:1 or 8:2) the reaction mixture was diluted with methanol and neutralized with prewashed Amberlite IRA 120-H^+. The resin was filtered off and the filtrate was concentrated under reduced pressure. The crude was purified by flash chromatography ($CHCl_3$ with gradient of methanol from 0 to 20%).

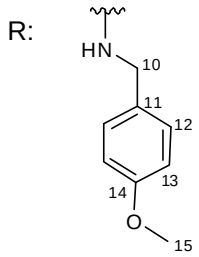


*General structure and numbering of pseudobimannoside bis-amides **2.2a-j** in the NMR characterizations*

2.4.7.2 N^1,N^2-bis(4-methoxybenzyl)amide, 2.2a

1,2-Cyclohexanedicarboxamides-N^1,N^2-bis(4-methoxybenzyl)-4-(2-azidoethoxy)-5-[α-D-mannopyranosyloxy]- (1*S*,2*S*,4*S*,5*S*)

Prepared according to general procedure 4, starting from **2.11a.**



2.2a

Yield = 73 %;

$[\alpha]_D^{20}$ = + 1.81 (c = 0.15 in ethanol)

MS (HRMS): calculated for: $[C_{32}H_{43}N_5O_{11}Na]^+$: 696.28568; found: 696.28465

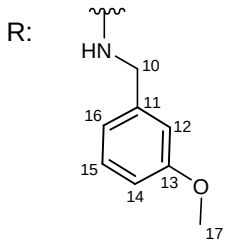
^{1}H NMR (400 MHz, CD_3OD): δ = 7.20 (d, 4H, H_{12}, $J_{12\text{-}13}$ = 8 Hz), 6.88 (d, 4H, H_{13}, $J_{13\text{-}12}$ = 8 Hz), 4.99 (d, 1H, $H_{1,}$), 4.26 (d, 4H, $H_{10a,b}$, $J_{10a\text{-}10b}$ = 2.4 Hz), 4.10 - 4.06 (m, 1H, D_2), 3.95 (dd, 1H, $H_{2,}$ $J_{2\text{-}1}$ = 1.6 Hz, $J_{2\text{-}3}$ = 3.2 Hz), 3.93 – 3.87 (m, 1H, H_{6a}), 3.86 – 3.69 (m, 11H, D_1, $H_{6b,}$ $H_{7a,b}$, H_3, H_{15}), 3.51 - 3.7 (m, 2H, $H_{4,}$ H_5), 3.49 – 3.35 (m, 2H, $H_{8a,b}$), 3.01 - 2.86 (m, 2H, D_4,D_5), 2.08 - 1.90 (m, 4H, D_3, D_6).

^{13}C NMR (100 MHz, CD_3OD): δ = 177.0, 176.8 (C_9); 160.4 (C_{14}); 132.2 (C_{11}); 129.9, 129.8 (C_{12}); 115.0 (C_{13}); 100.4 (C_1); 76.6 (C_3); 75.6 (C_5); 72.2 (C_{D1}); 72.5 (C_2); 72.5 (D_2); 69.3 (C_7); 68.9 (C_4); 63.2 (C_6); 55.8 (C_{15}); 52.2 (C_8); 43.6 (C_{10}); 42.1, 42.0 (C_{D4}, C_{D5}); 29.8, 29.0 (C_{D3}, C_{D6}).

2.4.7.3 N^1,N^2-bis(3-methoxybenzyl)amide, 2.2b

1,2-Cyclohexanedicarboxamides-N^1,N^2-bis(3-methoxybenzyl)-4-(2-azidoethoxy)-5-[α-D-mannopyranosyloxy]- (1*S*,2*S*,4*S*,5*S*)

Prepared according to general procedure 4, starting from **2.11b.**



2.2b

Yield = 67 %;

$[\alpha]_D^{20}$ = + 16.2 (c = 0.78 in methanol)

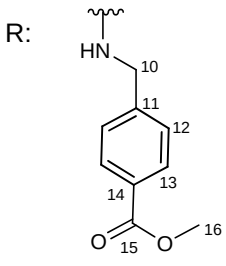
MS (HRMS): calculated for $[C_{32}H_{43}N_5O_{11}Na]^+$: 696.28568; found: 696.28599

^{1}H NMR (400 MHz, CD_3OD): δ = 7.20 (t, 2H, H_{15}, *J* = 8.0 Hz), 6.85 – 6.75 (m, 6H, H_{12}, H_{14}, H_{16}), 4.96 (br s, 1H, H_1), 4.28 (s, 4H, $H_{10a,b}$), 4.09 - 4.03 (m, 1H, D_2), 3.94 - 3.90 (m, 1H, H_2), 3.90 – 3.84 (m, 1H, H_{6a}), 3.83 – 3.65 (m, 11H, D_1, H_{17}, H_{6b}, $H_{7a,b}$, H_3), 3.64 - 3.54 (m, 2H, H_4, H_5), 3.47 – 3.31 (m, 2H, H_8), 3.03 - 2.85 (m, 2H, D_4, D_5), 2.08 - 1.90 (m, 4H, D_3, D_6).
^{13}C NMR (100 MHz, CD_3OD): δ = 177.2, 177.0 (C_9); 161.5 (C_{13}); 141.8 (C_{11}); 130.6 (C_{15}); 120.7, 120.7 (C_{16}); 114.0, 113.9, 113.7, 113,7 (C_{14}, C_{12}); 100.4 (C_1); 76.6 (C_3); 75.7 (C_5); 72.7 (C_{D1}); 72.5 (C_2); 72.4 (D_2); 69.3 (C_7); 68.9 (C_4); 63.2 (C_6); 55.8 (C_{17}); 52.1 (C_8); 44.0 (C_{10}); 42.1, 41.9 (C_{D4}, C_{D5}); 29.9, 29.0 (C_{D3}, C_{D6}).

2.4.7.4 *N*^{1},*N*^{2}-bis(4-carbomethoxybenzyl)amide, 2.2c

1,2-Cyclohexanedicarboxamides-*N*^{1},*N*^{2}-bis(4-carbomethoxy)-4-(2-azidoethoxy)-5-[α-D-mannopyranosyloxy]- (1*S*,2*S*,4*S*,5*S*)

Prepared according to general procedure 4, starting from **2.11c.**



2.2c

Yield = 60 %

$[\alpha]_D^{20}$ = +54.3 (c = 0.55 in methanol)

MS (HRMS) calculated for: $[C_{34}H_{43}N_5O_{13}Na]^+$: 752.27551; found: 752.27418

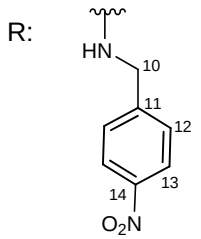
^{1}H NMR (400 MHz, CD_3OD): 7.81 (d, 4H, H_{13}, $J_{13\text{-}12}$ = 8 Hz), 7.28 (d, 4H, H_{12}, $J_{12\text{-}13}$ = 8 Hz), 4.96 (s, 1H, H_1), 4.51 - 4.21 (m, 4H, $H_{10a,b}$), 4.08 - 4.03 (m, 1H, D_2), 3.92 - 3.89 (m, 1H, H_2), 3.88 – 3.83 (m, 7H, H_{6a}, H_{16}), 3.83 – 3.64 (m, 5H, D_1, $H_{6b,}$ $H_{7a,b}$, $H_{3,}$), 3.62 - 3.52 (m, 2H, H_4, H_5), 3.45 – 3.33 (m, 2H, $H_{8a,b}$), 3.05 - 2.90 (m, 2H, D_4,D_5), 2.52 (s, 3H, H_{16}), 2.06 - 1.91 (m, 4H, D_3, D_6).

^{13}C NMR (100 MHz, CD_3OD): 177.5, 177.3 (C9); 168.5 (C_{15}); 145.9 (C_{11}); 130.8 (C_{13}); 130.0 (C_{14}); 128.2, 128.1 (C_{12}); 100.5 (C_1); 76.6 (C_3); 75.7 (C_5); 72.7 (C_{D1}); 72.6 (C_2); 72.5 (D_2); 69.3 (C_7); 68.9 (C_4); 63.2 (C_6); 52.7 (C_{16}); 52.2 (C_8); 43.6 (C_{10}); 42.0, 41.9 (C_{D4}, C_{D5}); 30.0, 29.2 (C_{D3}, C_{D6}).

2.4.7.5 N^1,N^2-bis(4-nitrobenzyl)amide, 2.2d

1,2-Cyclohexanedicarboxamides-N^1,N^2-bis(4-nitrobenzyl)-4-(2-azidoethoxy)-5-[α-D-mannopyranosyloxy]- (1*S*,2*S*,4*S*,5*S*)

Prepared according to general procedure 4, starting from **2.11d.**



2.2d

Yield = 72 %;

$[\alpha]_D^{20}$ = - 21.5 (c = 0.21 in methanol)

MS (HRMS) calculated for: $[C_{30}H_{37}N_7O_{13}Na]^+$: 726.23470; found: 726.23526

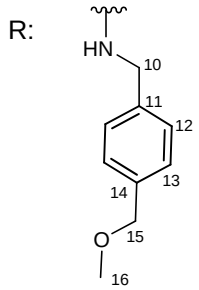
^{1}H NMR (400 MHz, CD_3OD): δ = 7.99 (d, 4H, H_{13}, $J_{13\text{-}12}$ = 7.4 Hz), 7.40 (d, 4H, H_{12}, $J_{12\text{-}13}$ = 7.4 Hz), 4.99 (s, 1H, H_1), 4.60 (d, 2H, H_{10a}, $J_{10a\text{-}10b}$ = 16.4 Hz), 4.30 (d, 2H, H_{10b}, $J_{10b\text{-}10a}$ = 16.4 Hz), 4.10 - 4.06 (m, 1H, D_2), 3.94 - 3.91 (m, 1H, H_2), 3.89 – 3.84 (m, 1H, H_{6a}), 3.84 – 3.66 (m, 5H, D_1, H_{6b}, $H_{7a,b}$, H_3,), 3.65 - 3.56 (m, 2H, H_4, H_5), 3.49 - 3.35 (m, 2H, $H_{8a,b}$), 3.10 - 2.95 (m, 2H, D_4,D_5), 2.10 - 1.92 (m, 4H, D_3, D_6).

^{13}C NMR (100 MHz, CD_3OD): δ = 177.8, 177.6 (C_9); 148.3 (C_{14}); 148.1 (C_{11}); 128.8 (C_{12}); 124.5 (C_{13}); 100.6 (C_1); 76.6 (C_3); 75.7 (C_5); 72.8 (C_{D1}); 72.6 (C_2); 72.6 (C_{D2}); 69.4 (C_7); 68.9 (C_4); 63.2 (C_6); 52.2 (C_8); 43.3 (C_{10}); 41.9, 41.8 (C_{D4}, C_{D5}); 30.1, 29.3 (C_{D3}, C_{D6}).

2.4.7.6 N^1,N^2-bis(4-(methoxymethylene)benzyll)amide, 2.2e

1,2-Cyclohexanedicarboxamides-N^1,N^2-bis(4-methoxybenzyl)-4-(2-azidoethoxy)-5-[α-D-mannopyranosyloxy]- (1*S*,2*S*,4*S*,5*S*)

Prepared according to general procedure 4, starting from **2.11e.**



2.2e

Yield = 78 %;

[α]D20 = + 5.2 (c = 0.22 in methanol)

MS (HRMS) calculated for: $[C_{34}H_{47}N_5O_{11}Na]^+$: 724.31698; found: 724.31565

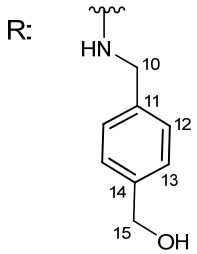
^{1}H NMR (400 MHz, CD_3OD): δ = 7.28 – 7.18 (m, 8H, H_{12}, H_{13}), 4.94 (br s, 1H, H_1), 4.40 (s, 4H, H_{15}), 4.25 (br s, 4H, H_{10}), 4.06-4.01 (m, 1H, D_2), 3.91 - 3.87 (m, 1H, H_2), 3.87 – 3.82 (m, 1H, H_{6a}), 3.80 – 3.64 (m, 5H, D_1, H_{6b}, $H_{7a,b}$, H_3,), 3.61 - 3.54 (m, 2H, H_4, H_5), 3.44 – 3.32 (m, 8H, $H_{8a,b}$, H_{16}), 3.98 - 2.83 (m, 2H, D_4,D_5), 2.02 - 1.87 (m, 4H, D_3, D_6).

^{13}C NMR (100 MHz, CD_3OD): δ = 177.2, 177.0 (C_9); 139.8 (C_{14}); 138.4, 138.3 (C_{11}); 129.3 (C_{13}); 128.6, 128.5 (C_{12}); 100.4 (C_1); 76.6 (C_3); 75.7 (C_5); 75.5 (C_{15}); 72.7 (C_{D1}); 72.5 (C_2); 72.4 (D_2); 69.3 (C_7); 68.9 (C_4); 63.2 (C_6); 58.4 (C_{16}); 52.1 (C_8); 43.8 (C_{10}); 42.1, 41.9 (C_{D4}, C_{D5}); 29.9, 29.0 (C_{D3}, C_{D6}).

2.4.7.7 N^1,N^2-bis(4-(hydroxymethylene)benzyl)amide, 2.2f

1,2-Cyclohexanedicarboxamides-N^1,N^2-bis(4-hydroxymethylenebenzyl)-4-(2-azidoethoxy)-5-[α-D-mannopyranosyloxy]- (1*S*,2*S*,4*S*,5*S*)

Prepared according to general procedure 4, starting from **2.11f.**



2.2f

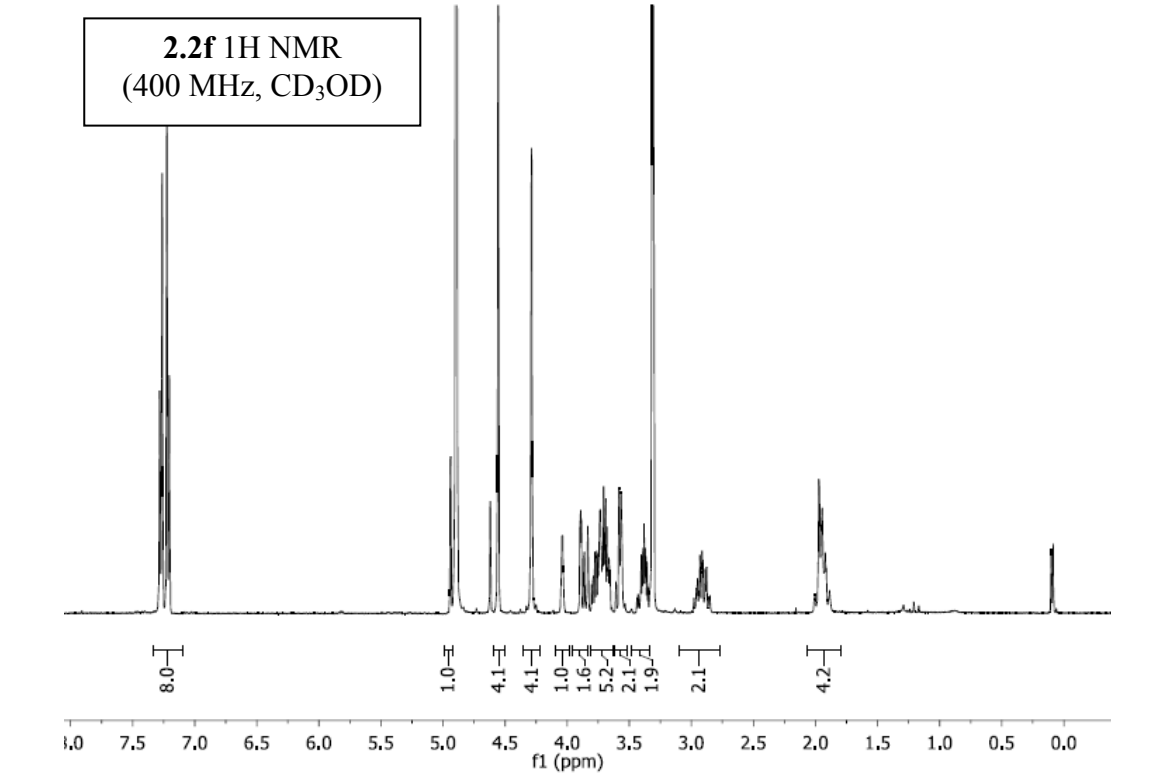
Yield = 82 %;

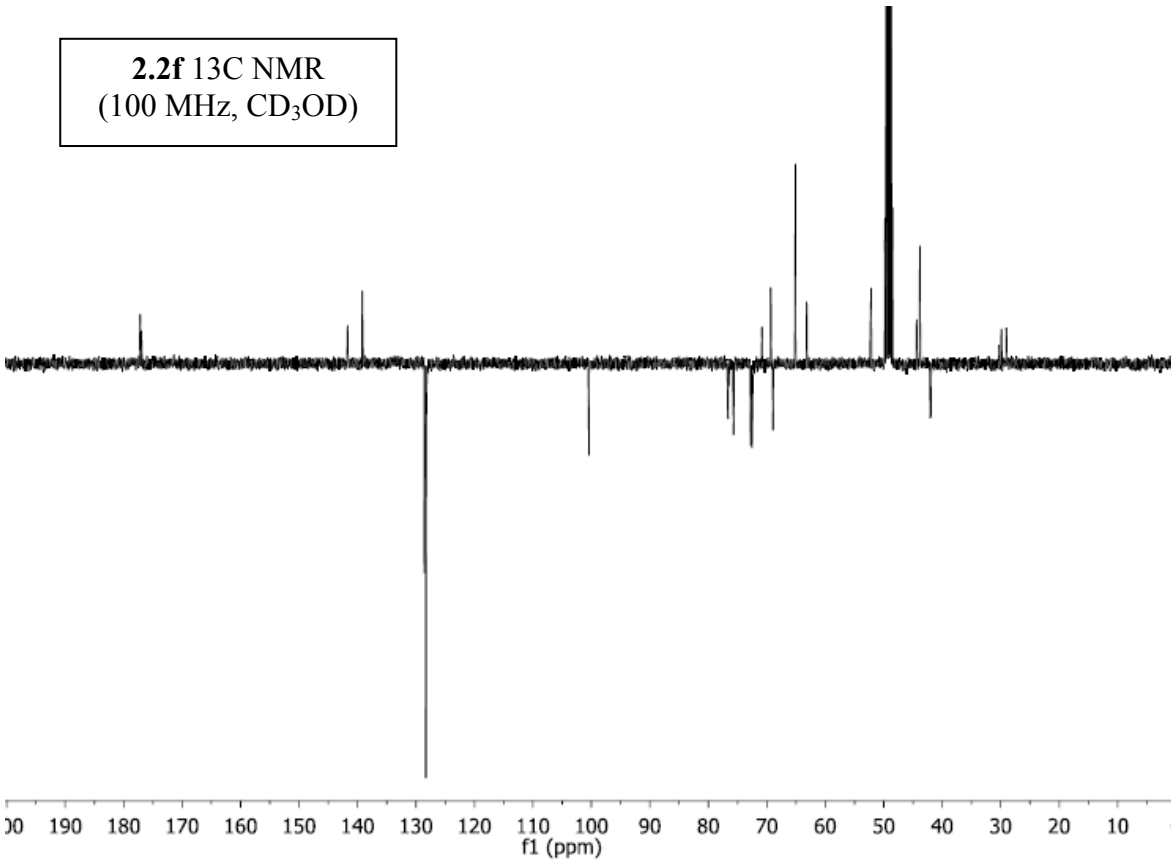
$[\alpha]_D^{20}$ = + 12.1 (c = 0.81 in methanol)

MS (HRMS) calculated for: $[C_{32}H_{43}N_5O_{11}Na]^+$: 696.28568; found: 696.28423.

^{1}H NMR (400 MHz, CD_3OD): δ = 7.29 (d, 4H, H_{12}, $J_{12\text{-}13}$ = 8 Hz), 7.23 (d, 4H, H_{13}, $J_{13\text{-}12}$ = 8 Hz), 4.96 (d, 1H, H_1, $J_{1\text{-}2}$ = 1,6 Hz), 4,58 (s, 4H, $H_{15a,b}$), 4.31 (s, 4H, $H_{10a,b}$), 4.08 - 4.03 (m, 1H, D_2), 3.93 - 3.89 (m, 1H, H_2), 3.89 – 3.84 (m, 1H, H_{6a}), 3.84 – 3.65 (m, 5H, D_1, $H_{6b,}$ $H_{7a,b}$, $H_{3,}$), 3.64 - 3.54 (m, 2H, H_4, H_5), 3.47 – 3.35 (m, 2H, $H_{8a,b}$), 3.02 - 2.85 (m, 2H, D_4,D_5), 2.06 - 1.86 (m, 4H, D_3, D_6).

^{13}C NMR (100 MHz, CD_3OD): δ = 177.2, 177.0 (C_9); 141.7 (C_{14}); 139.2 (C_{11}); 128.6, 128.7 (C_{12}); 128.3 (C_{13}); 100.4 (C_1); 76.6 (C_3); 75.7 (C_5); 72.7 (C_{D1}); 72.5 (C_2); 72.4 (D_2); 69.3 (C_7); 68.9 (C_4); 65.1 (C_{15}); 63.2 (C_6); 52.1 (C_8); 43.8 (C_{10}); 42.1, 41.9 (C_{D4}, C_{D5}); 29.9, 29.0 (C_{D3}, C_{D6}).

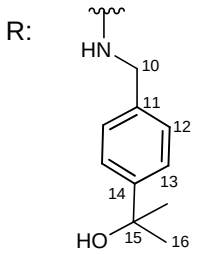




2.4.7.8 N^1,N^2-bis(4-(hydroxy(α,α-dimethyl)methylen)benzyl)amide, 2.2g

1,2-Cyclohexanedicarboxamides-N^1,N^2-bis(4-(hydroxy(α,α-dimethyl)methylen)benzyl)-4-(2-azidoethoxy)-5-[α-D-mannopyranosyloxy]- (1*S*,2*S*,4*S*,5*S*)

Prepared according to general procedure 4, starting from **2.11g.**



2.2g

Yield = 65 %;

[α]D20 = + 5.3 (c = 0.48 in methanol)

MS (HRMS) calculated for: $[C_{36}H_{51}N_5O_{11}Na]^+$: 752.34828; found: 752. 34702.

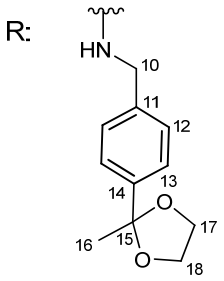
^{1}H NMR (400 MHz, CD_3OD): δ = 7.40 (d, 4H, H_{12}, $J_{12\text{-}13}$ = 8.1 Hz), 7.18 (d, 4H, H_{13}, $J_{13\text{-}12}$ = 8.1 Hz), 4.93 (br s, 1H, H_1), 4.26 (s, 4H, $H_{10a,b}$), 4.04 - 3.00 (m, 1H, D_2), 3.88 - 3.86 (m, 1H, H_2), 3.86 – 3.79 (m, 1H, H_{6a}), 3.78 – 3.61 (m, 5H, D_1, H_{6b}, $H_{7a,b}$, H_3,), 3.59 - 3.50 (m, 2H, H_5, H_4,), 3.43 – 3.32 (m, 2H, $H_{8a,b}$), 2.98 - 2.82 (m, 2H, D_4,D_5), 2.00 - 1.84 (m, 4H, D_3, D_6), 1.47 (s, 12H, H_{16}).

^{13}C NMR (100 MHz, CD_3OD): δ = 177.1, 176.9 (C_9); 149.9 (C_{14}); 138.2 (C_{11}); 128.3, 128.2 (C_{12}); 125.9 (C_{13}); 100.4 (C_1); 76.6 (C_3); 75.7 (C_5); 73.0 (C_{15}); 72.7 (C_{D1}); 72.5 (C_2); 72.4 (D_2); 69.3 (C_7); 68.9 (C_4); 63.2 (C_6); 52.1 (C_8); 43.8 (C_{10}); 42.1, 42.0 (C_{D4}, C_{D5}); 32.1 (C_{16}); 29.9, 29.0 (C_{D3}, C_{D6}).

2.4.7.9 N^1,N^2-bis(4-(2-methyl-1,3-dioxolan-2-yl)benzyl)amide, 2.2h

1,2-Cyclohexanedicarboxamides-N^1,N^2-bis(4-(2-methyl-1,3-dioxolan-2-yl)-4-(2-azidoethoxy)-5-[α-D-mannopyranosyloxy]- (1*S*,2*S*,4*S*,5*S*)

Prepared according to general procedure 4, starting from **2.11h.**



2.2h

Yield = 58 %;

MS (ESI) calculated for: $[C_{38}H_{51}N_5O_{13}Na]^+$: 808.8; found: 808.4

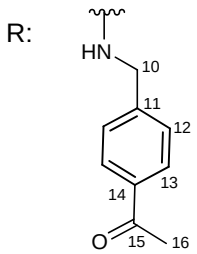
^{1}H NMR (400 MHz, CD_3OD): δ = 7.38 (d, 4H, H_{13}, $J_{12\text{-}13}$ = 7.7 Hz), 7.21 (d, 4H, H_{12}, $J_{12\text{-}13}$ = 7.7 Hz), 4.94 (d, 1H, H_1, $J_{1\text{-}2}$ = 1.4 Hz), 4.34 – 4.20 (m, 4H, H_{10}), 4.06-4.01 (m, 4H, H_{17a}, H_{18a}), 4.00 - 3.95 (m, 1H, D_2), 3.91 - 3.87 (m, 1H, H_2), 3.87 – 3.82 (m, 1H, H_{6a}), 3.80 – 3.62 (m, 9H, D_1, H_{6b}, $H_{7a,b}$, H_3, H_{17b}, H_{18b}), 3.61 - 3.54 (m, 2H, H_4, H_5), 3.44 – 3.32 (m, 2H, $H_{8a,b}$), 3.98 - 2.83 (m, 2H, D_4,D_5), 2.02 - 1.87 (m, 4H, D_3, D_6), 1.54 (s, 6H, H_{16}).

^{13}C NMR (100 MHz, CD_3OD): δ = 177.2, 177.0 (C_9); 143.7 (C_{11}); 139.9 (C_{14}); 128.5, 128.4 (C_{12}); 126.6, (C_{13}); 100.4 (C_1); 76.6 (C_3); 75.7 (C_5); 72.7, 72.6, 72.5 (C_2, C_{D1}, D_2); 69.3 (C_7); 68.9 (C_4); 65.6 (C_{17}, C_{18}); 63.2 (C_6); 58.4 (C_{16}); 52.2 (C_8); 43.8 (C_{10}); 42.1, 42.0 (C_{D4}, C_{D5}); 29.0, 29.0 (C_{D3}, C_{D6}); 28.1 (C_{16}).

2.4.7.10 *N*^{1},*N*^{2}-bis(4-acetylbenzyl)amide, 2.2i

1,2-Cyclohexanedicarboxamides-*N*^{1},*N*^{2}-bis(4-acetylbenzyl)-4-(2-azidoethoxy)-5-[α-D-mannopyranosyloxy]- (1*S*,2*S*,4*S*,5*S*)

Prepared according to general procedure 4, starting from **2.11i.**



2.2i

Yield: 83%

$[\alpha]_D^{20}$ = - 42.8 (c = 0.1 in methanol)

MS (HRMS) calculated for: $[C_{34}H_{43}N_5O_{11}Na]^+$: 720.28568; found: 720.28552

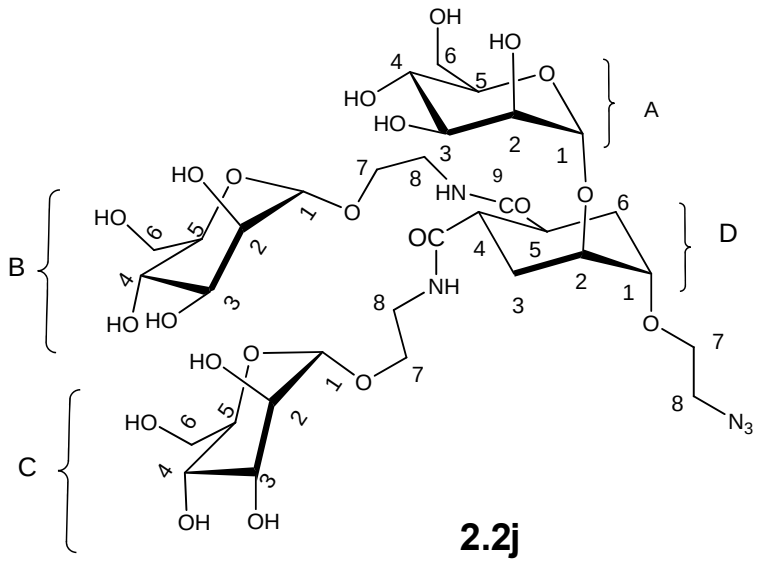
^{1}H NMR (400 MHz, CD_3OD): δ = 7.82 (d, 4H, H_{13}, $J_{13\text{-}12}$ = 7.5 Hz), 7.33 (d, 4H, H_{12}, $J_{12\text{-}13}$ = 7.5 Hz), 4.98 (d, 1H, H_1, $J_{1\text{-}2}$ = 1.6 Hz), 4.54 - 4.26 (m, 4H, $H_{10a,b}$), 4.10 - 4.05 (m, 1H, D_2), 3.94 - 3.90 (m, 1H, H_2), 3.90 – 3.84 (m, 1H, H_{6a}), 3.84 – 3.66 (m, 5H, D_1, $H_{6b,}$ $H_{7a,b}$, H_3), 3.64 - 3.57 (m, 2H, H_5, H_4), 3.47 – 3.35 (m, 2H, $H_{8a,b}$), 3.08 - 2.92 (m, 2H, D_4,D_5), 2.52 (s, 3H, H_{16}), 2.52 (s, 3H, H_{16}), 2.08 - 1.92 (m, 4H, D_3, D_6).

^{13}C NMR (100 MHz, CD_3OD): δ = 200.2 (C_{15}); 177.5, 177.3 (C_9); 146.2 (C_{14}); 137.1 (C_{11}); 129.8 (C_{13}); 128.3 (C_{12}); 100.5 (C_1); 76.6 (C_3); 75.7 (C_5); 72.8 (C_{D1}); 72.6 (C_2); 72.6 (D_2); 69.4 (C_7); 68.9 (C_4); 63.2 (C_6); 52.2 (C_8); 43.6 (C_{10}); 42.0, 41.9 (C_{D4}, C_{D5}); 30.0, 29.2 (C_{D3}, C_{D6}); 26.8 (C_{16}).

2.4.7.11 N^1,N^2-bis(2-(α-D-mannopyranosyloxy)ehyl)amide, 2.2j

1,2-Cyclohexanedicarboxamides-N^1,N^2-bis(2-(α-D-mannopyranosyloxy)ethyl)-4-(2-azidoethoxy)-5-[α-D-mannopyranosyloxy]- (1*S*,2*S*,4*S*,5*S*)

Prepared according to general procedure 4, starting from **2.11j.**



2.2j

Yield = 85 %;

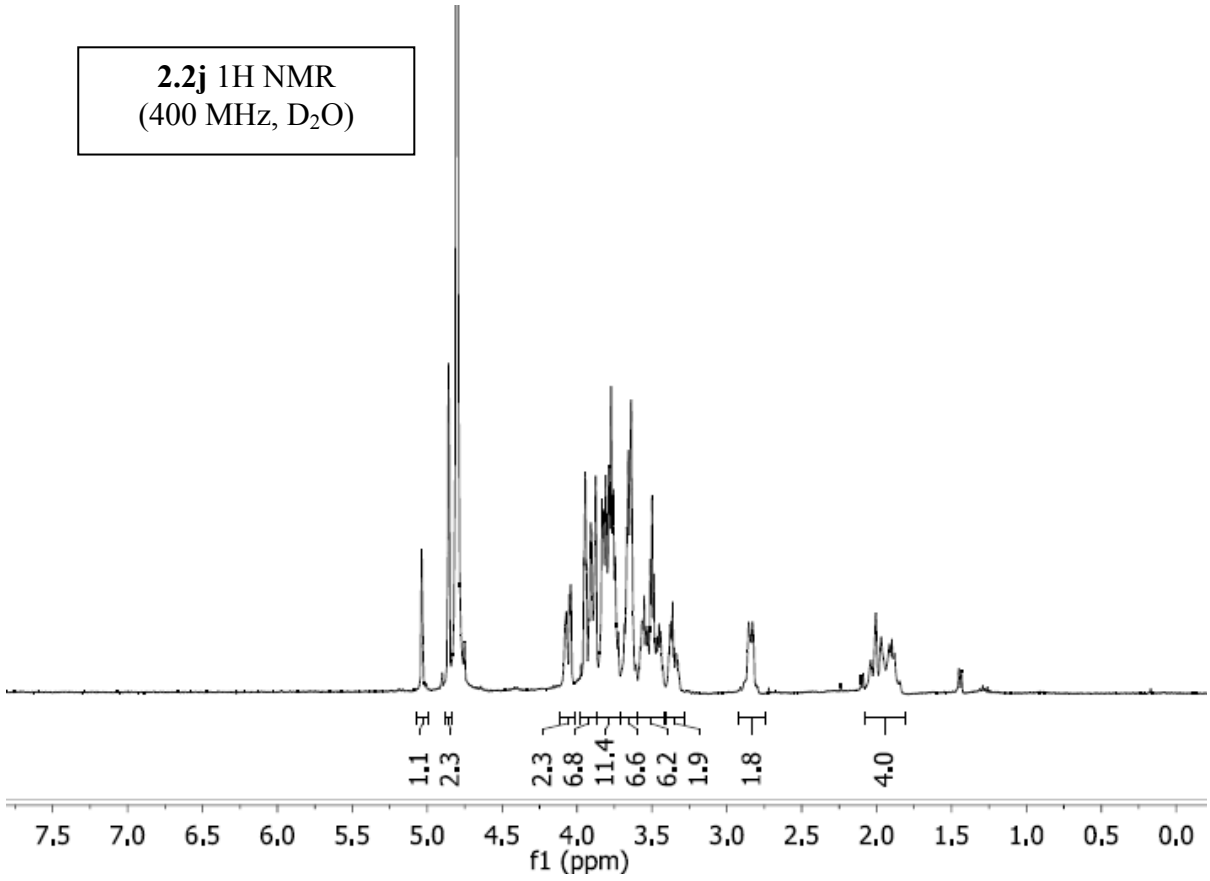
$[\alpha]_D^{20}$ = + 48.4 (c = 0.45 in H_2O);

MS (ESI): calculated for: $[C_{32}H_{55}N_5O_{21}Na]^+$: 868.8; found: 868.4

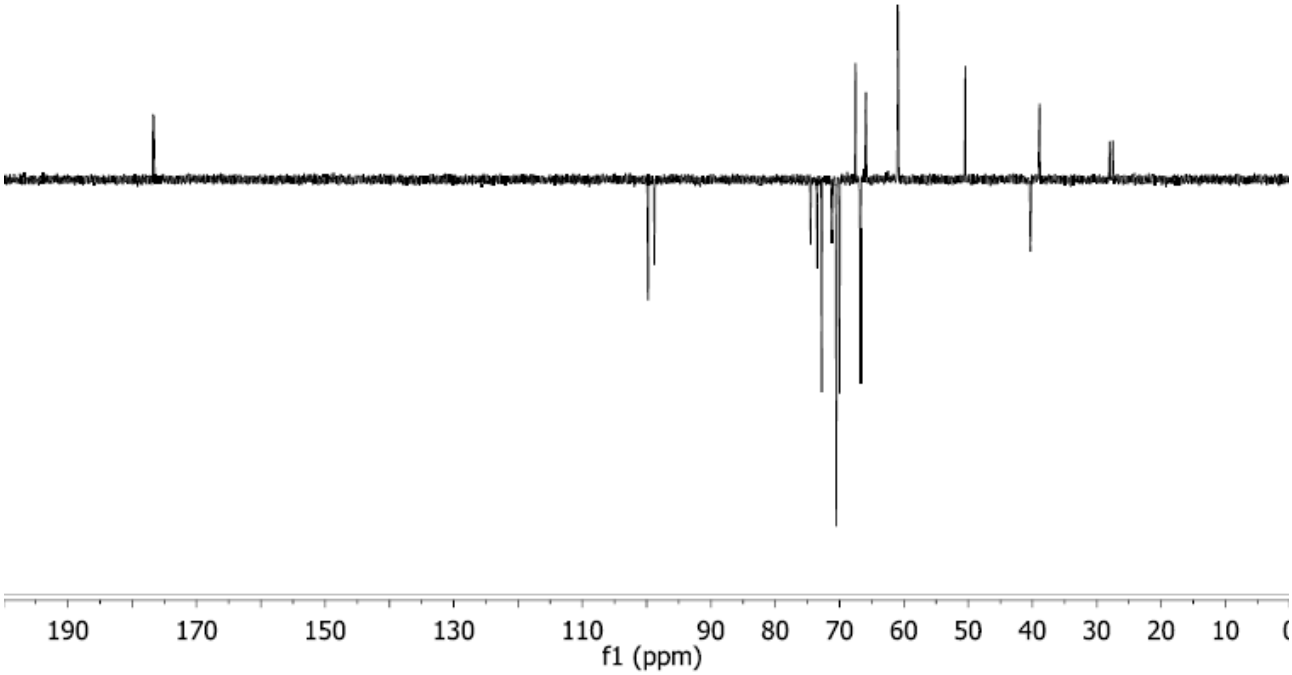
^{1}H NMR (400 MHz, D_2O): δ = 5.03 (d, 1H, A_1, $J_{1\text{-}2}$ = 1.6 Hz), 4.86 (s, 2H, B_1, C_1, $J_{1\text{-}2}$ = 1.6 Hz), 4.10 - 4.02 (m, 2H, A_2, D_2), 3.97 – 3.92 (m, 2H, B_2, B_2), 3.92 – 3.86 (m, 4H, A_3, A_{6a}, B_{6a},

C_{6a}), 3.85 - 3.71 (m, 10H, D_1, B_3, C_3, A_{6b}, B_{6b}, C_{6b}, H_7, B_{7a}, C_{7a}), 3.70 - 3.59 (m, 6H, A_4, A_5, B_4, B_5, C_4, C_5), 3.59 - 3.52 (m, 2H, , B_{7b}, C_{7b}), 3.50 (t, 2H, H_8, $J_{7\text{-}8}$ = 4.76 Hz), 3.47 - 3.41 (m, 2H, B_{8a}, C_{8a}), 3.40 - 3.30 (m, 2H, B_{8b}, C_{8b}), 2.92 - 2.78 (m, 2H, D_4, D_5), 2.07 - 1.81 (m, 4H, D_3, D_6).

^{13}C NMR (100 MHz, D_2O): δ = 176.8, 176.6 (C_9); 99.8, 99.8 (C_{B1}, C_{C1}); 98.8 (C_{A1}); 74.5 (C_{D1}); 73.5 (C_{A5}); 72.8 (C_{B5}, C_{C5}); 71.2 (C_{D2}); 70.5 (C_{A2}, C_{A3}, C_{B3}, C_{C3}); 70.0 (C_{B2}, C_{C2}); 67.5 (C_7); 66.8 (C_{A4}); 66.7 (C_{C4}, C_{D4}); 65.9 (C_{B7}, C_{C7}); 61.0 (C_{A6}); 60.9 (C_{B6}, C_{C6}); 50.5 (C_8); 40.3, 40.3 (C_{D4}, C_{D5}); 38.9 (C_{B8}, C_{C8});; 28.0, 27.5 (C_{D3}, C_{D6}).



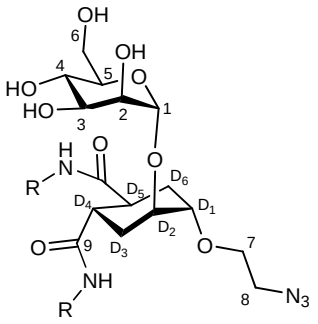
2.2j 13C NMR
(100 MHz, D_2O)



2.4.8 Synthesis and characterization of final DC-SIGN ligands 1,2-Cyclohexanedicarboxamide, 4-(2-azidoethoxy)-5-(α-D-mannopyranosyloxy),- (1*S*,2*S*,4*S*,5*S*), 2.2k-s

2.4.8.1 General procedure 5

The amine **2.12** (3 eq) was added to a 0.1 M PNP-scaffold **2.31** (1 eq) in dry MeCN under stirring and under nitrogen atmosphere at room temperature. After completion (TLC, hex : EtOAc) the solvent was evaporated under reduced pressure. The crude product was dissolved in dry methanol (c = 0.1 M), under nitrogen at room temperature, and a 1M solution of sodium methoxide in MeOH (2 eq) was added. After reaction completion the reaction mixture was diluted with methanol and neutralized with prewashed Amberlite IRA 120-H^+. The resin was filtered off and the filtrate was concentrated under reduced pressure. The crude was purified by flash chromatography ($CHCl_3$ with gradient of methanol from 0 to 20%).

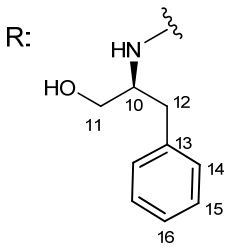


*General structure and numbering of pseudobimannoside bis-amides **2.10a-j** in the NMR characterizations of*

2.4.8.2 N^1,N^2-bis((L)phenylalaninol)amide, 2.2k

1,2-Cyclohexanedicarboxamides-N^1,N^2-bis((L)phenylalaninol)-4-(2-azidoethoxy)-5-[α-D-mannopyranosyloxy]- (1*S*,2*S*,4*S*,5*S*)

Prepared according to general procedure 5 using amine **2.12k.**



2.2k

Yield = 75 %;

$[\alpha]_D^{20}$ = + 17.8 (c = 0.5 in methanol);

MS (HRMS): calculated for: $[C_{34}H_{47}N_5O_{11}Na]^+$: 724.31698; found: 724.31698

^{1}H NMR (400 MHz, CD_3OD): δ = 7.31 - 7.10 (m, 10H, H_{14}, H_{15}, H_{16}), 4.96 (br s, 1H, H_1), 4.12 - 4.00 (m, 2H, H_{10}), 3.88 (dd, 1H, H_2, $J_{2\text{-}1}$ = 1.6 Hz, $J_{2\text{-}3}$ = 3.1 Hz), 3.90 – 3.80 (m, 2H, H_5, H_{6b}), 3.72. - 3.63 (m, 4H, D_2, H_{6a}, H_7), 3.63 - 3.59 (m, 2H, H_4, D_1), 3.58 - 3.54 (m, 1H, H_3), 3.47 (d, 4H, H_{11}, $J_{11\text{-}10}$= 5.4), 3.39 - 3.33 (m, 2H, H_8), 3.00 - 2.88 (m, 2H, H_{12}), 2.71 - 2.57 (m, 4H, H_{11}, D_4, D_5), 1.63 - 1.40 (m, 4H, D_3, D_6).

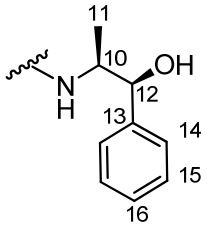
^{13}C NMR (100 MHz, CD_3OD): δ = 177.2, 177.1 (C_9); 140.1 (C_{13}); 130.6, 129.5, 129.4, 127.4 (C_{14}, C_{15}, C_{16}); 100.2 (C_1); 76.5 (C_3); 75.6 (C_{D1}); 72.8 (C_{D2}); 72.6 (C_5); 72.1 (C_2); 69.3 (C_7); 68.8

(C_4); 64.8 (C_{11}); 63.2 (C_6); 53.9 (C_{10}); 52.1 (C_8); 41.9, 41.7 (C_{D4}, C_{D5}); 38.1 (C_{12}); 29.9.5, 28.7 (C_{D3}, C_{D6}).

2.4.8.3 N^1,N^2-bis((1*S*,2*S*)-2-amino-1-phenylpropan-1-ol)amide, 2.2l

1,2-Cyclohexanedicarboxamides-N^1,N^2-bis((1*S*,2*S*)-2-amino-1-phenylpropan-1-ol)-4-(2-azidoethoxy)-5-[α-D-mannopyranosyloxy]- (1*S*,2*S*,4*S*,5*S*)

Prepared according to general procedure 5 using amine **2.12l.**



2.2l

Yield: 75 %

$[\alpha]_D^{20}$ = + 17.8 (c = 0.5 in methanol)

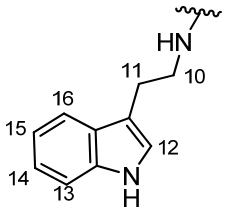
MS (HRMS): calculated for: $[C_{34}H_{47}N_5O_{11}Na]^+$: 724.31698; found: 724.31698

^{1}H NMR (400 MHz, CD_3OD): δ = 7.36 (d, 4H, H_{14}, $J_{14\text{-}15}$ = 7.2 Hz), 7.27 (t, 4H, H_{15}, $J_{14\text{-}15}$ = 7.2 Hz), 7.18 (t, 2H, H_{16}, $J_{14\text{-}15}$ = 7.2 Hz), 4.96 (br s, 1H, H_1), 4.80 (d, 2H, H_{12}, $J_{12\text{-}10}$ = 1.7 Hz), 4.12 - 4.00 (m, 3H, H_{D2}, H_{10}), 3.94 (dd, 1H, H_2, $J_{2\text{-}1}$ = 1.6 Hz, $J_{2\text{-}3}$ = 3.1 Hz), 3.90 – 3.80 (m, 2H, H_5, H_{6b}), 3.82 - 3.63 (m, 5H, D_2, H_3, H_{6a}, $H_{7a,b}$), 3.63 - 3.59 (m, 2H, H_4, D_1), 3.49 – 3.36 (m, 2H, $H_{8a,b}$), 2.97 – 2.822 (m, 2H, D_4,D_5), 1.96 - 1.84 (m, 4H, D_3, D_6), 0.95 (t, 6H, H_{11}, $J_{11\text{-}10}$ = 6.4 Hz), **^{13}C NMR** (100 MHz, CD_3OD): δ = 177.2, 177.1 (C_9); 143.4, 143.3 (C_{14}, C_{15}); 129.2 (C_{15}), 125.2 (C_{16}), 127.4 (C_{14}); 100.5 (C_1); 76.7 (C_3); 76.1, 76.0 (C_{12}, C_{D1}); 75.7 (C_5); 72.8 (C_{D1}); 72.5, 72.5 (C_2, D_2); 69.4 (C_7); 68.9 (C_4); 63.2 (C_6); 52.5 (C_8); 52.2 (C_{10}); 42.1, 41.9 (C_{D4}, C_{D5}); 30.1, 29.1 (C_{D3}, C_{D6}); 13.4, 13.3 (C_{11}).

2.4.8.4 N^1,N^2-bis(2-(1H-indol-3-yl)ethyl)amide, 2.2m

1,2-Cyclohexanedicarboxamides-N^1,N^2-bis(2-(1H-indol-3-yl)ethanamine)-4-(2-azidoethoxy)-5-[α-D-mannopyranosyloxy]- (1*S*,2*S*,4*S*,5*S*)

Prepared according to general procedure 5 using amine **2.12m.**



2.2m

Yield = 47 %;

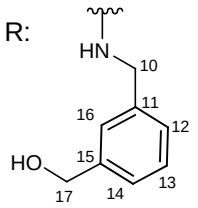
$[\alpha]_D^{20}$ = + 38.6 (c = 0.575 in methanol)

^{1}H NMR (400 MHz, CD_3OD): δ = 2.54 (d, 2H, $H_{16,}$ $J_{16\text{-}15}$ = 7.9 Hz), 7.3 (dd, 2H, $H_{13,}$ $J_{13\text{-}14}$ = 8.1 Hz, $J_{13\text{-}15}$ = 0.8 Hz), 7.10 – 7.03 (m, 4H, H_{14}, H_{12}), 7.00 – 6.95 (m, 2H, H_{15}), 4.92 (d, 1H, H_1, $J_{1\text{-}2}$ = 1,6 Hz), 4.01 - 3.96 (m, 1H, D_2), 3.90 – 3.83 (m, 2H, H_2, H_{6a}), 3.75 – 3.52 (m, 9H, D_1, $H_{6b,}$ $H_{7a,b}$, H_3, H_{10}), 3.51 – 3.32 (m, 4H, $H_{8a,b}$, H_4, H_5), 2.96 - 2.81 (m, 4H, H_{10}), 2.80 – 2.66 (m, 2H, D_4,D_5), 1.94 - 1.70 (m, 4H, D_3, D_6).

2.4.8.5 N^1,N^2-bis(3-(hydroxymethylene)benzyl)amide, 2.2n

1,2-Cyclohexanedicarboxamides-N^1,N^2-bis(3-(hydroxymethylene)benzyl)-4-(2-azidoethoxy)-5-[α-D-mannopyranosyloxy]- (1*S*,2*S*,4*S*,5*S*)

Prepared according to general procedure 5 using amine **2.12n.**



2.2n

Yield = 65 %;

$[\alpha]_D^{20}$ = + 21.5 (c = 0.33 in methanol);

MS (HRMS) calculated for: $[C_{32}H_{43}N_5O_{11}Na]^+$: 696.28568; found: 696.28578

^{1}H NMR (400 MHz, CD_3OD): δ = 7.35 – 7.18 (m, 8H, H_{12}, H_{13}, H_{14}, H_{16}), 5.01 (d, 1H, H_1, $J_{1\text{-}2}$ = 1,6 Hz), 4,61 (s, 4H, $H_{17a,b}$) 4.41 – 4.31 (m, 4H, $H_{10a,b}$), 4.13 - 4.08 (m, 1H, D_2), 3.96 (dd, 1H, $H_{2,}$ $J_{2\text{-}1}$ = 1.6 Hz, $J_{2\text{-}3}$ = 3.2 Hz), 3.94 – 3.89 (m, 1H, H_{6a}), 3.88 – 3.71 (m, 5H, D_1, $H_{6b,}$ $H_{7a,b}$, $H_{3,}$),

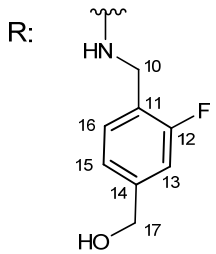
3.71 - 3.59 (m, 2H, H_4, H_5), 3.51 – 3.38 (m, 2H, $H_{8a,b}$), 3.06 – 2.92 (m, 2H, D_4,D_5), 2.09 - 1.95 (m, 4H, D_3, D_6).

^{13}C NMR (100 MHz, CD_3OD): δ = 177.2, 177.0 (C_9); 143.1 (C_{15}); 140.3 (C_{11}); 129.7 (C_{16}); 127.7, 127.1, 126.8 (C_{12}, C_{13}, C_{14}); 100.5 (C_1); 76.7 (C_3); 75.7 (C_5); 72.8 (C_{D1}); 72.6 (C_2, D_2); 69.3 (C_7); 69.0 (C_4); 65.3 (C_{17}); 63.2 (C_6); 52.2 (C_8); 44.0 (C_{10}); 42.1, 41.9 (C_{D4}, C_{D5}); 29.9, 29.1 (C_{D3}, C_{D6}).

2.4.8.6 *N*^{1},*N*^{2}-bis(2-fluoro-4-(hydroxymethylene)benzyl)amide, 2.2o

1,2-Cyclohexanedicarboxamides-*N*^{1},*N*^{2}-bis(2-fluoro-4-(hydroxymethylene)benzyl)-4-(2-azidoethoxy)-5-[α-D-mannopyranosyloxy]- (1*S*,2*S*,4*S*,5*S*)

Prepared according to general procedure 5 using amine **2.12o.**



2.2o

Yield = 76 %

$[\alpha]_D^{20}$ = + 9.3 (c = 0.44 in methanol)

MS (HRMS) calculated for: $[C_{32}H_{41}F_2N_5O_{11}Na]^+$: 732.26683; found: 732.26691.

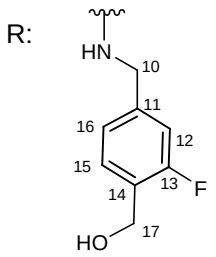
^{1}H NMR (400 MHz, CD_3OD): δ = 7.22 (t, 2H, H_{16}, $J_{16\text{-}15}$ = 7.7 Hz, $J_{16\text{-}F}$ = 7.7 Hz), 7.05 (d, 2H, H_{13}, $J_{13\text{-}F}$ = 11.5 Hz), 7.04 (d, 2H, H_{15}, $J_{15\text{-}16}$ = 7.7 Hz), 4.94 (br s, 1H, H_1), 4,55 (s, 4H, $H_{17a,b}$), 4.37 – 4.26 (m, 4H, $H_{10a,b}$), 4.04 - 4.00 (m, 1H, D_2), 3.89 (dd, 1H, H_2, $J_{2\text{-}1}$ = 1.6 Hz, $J_{2\text{-}3}$ = 3.1 Hz), 3.87 – 3.81 (m, 1H, H_{6a}), 3.81 – 3.63 (m, 5H, D_1, $H_{6b,}$ $H_{7a,b}$, $H_{3,}$), 3.62 - 3.51 (m, 2H, H_4, H_5), 3.44 – 3.31 (m, 2H, $H_{8a,b}$), 2.99 - 2.83 (m, 2H, D_4,D_5), 2.01 - 1.86 (m, 4H, D_3, D_6).

^{13}C NMR (100 MHz, CD_3OD): δ = 177.3, 177.1 (C_9); 162.1 (d, C_{12}, $J_{12\text{-}F}$ = 250 Hz); 144.8, 144.7 (d, C_{14}, $J_{14\text{-}F}$ = 4.3 Hz); 130.6, 130.5 (d, C_{16}, $J_{16\text{-}F}$ = 4.5 Hz); 125.5 (d, C_{11}, $J_{11\text{-}F}$ = 13.8 Hz); 123.5 (d, C_{15}, $J_{15\text{-}F}$ = 3.1 Hz); 114.3 (d, C_{13}, $J_{13\text{-}F}$ = 22.1 Hz); 100.4 (C_1); 76.6 (C_3); 75.7 (C_5); 72.7 (C_{D1}); 72.5 (C_2); 72.5 (D_2); 69.3 (C_7); 68.9 (C_4); 64.3 (C_{17}); 63.1 (C_6); 52.1 (C_8); 42.0, 41.8 (C_{D4}, C_{D5}); 37.7 (d, C_{10}, $J_{10\text{-}F}$ = 4.6 Hz); 29.8, 29.0 (C_{D3}, C_{D6}).

2.4.8.7 N^1,N^2-bis(3-fluoro-4-(hydroxymethylene)benzyl)amide, 2.2p

1,2-Cyclohexanedicarboxamides-N^1,N^2-bis(3-fluoro-4-(hydroxymethylene)benzyl)-4-(2-azidoethoxy)-5-[α-D-mannopyranosyloxy]- (1*S*,2*S*,4*S*,5*S*)

Prepared according to general procedure 5 using amine **2.12p.**



2.2p

Yield = 64 %

$[\alpha]_D^{20}$ = + 6.9 (c = 0.33 in methanol)

MS (HRMS) calculated for: $[C_{32}H_{41}F_2N_5O_{11}Na]^+$: 732.26683; found: 732.26529.

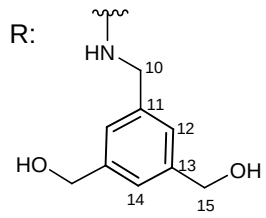
^{1}H NMR (400 MHz, CD_3OD): δ = 77.22 (t, 2H, H_{15}, $J_{15\text{-}16}$ = 7.7 Hz, $J_{15\text{-}F}$ = 7.7 Hz), 7.12 (d, 2H, H_{16}, $J_{16\text{-}15}$ = 7.7 Hz), 7.04 (d, 2H, H_{12}, $J_{12\text{-}F}$ = 11.5 Hz), 5.04 (d, 1H, H_1, $J_{1\text{-}2}$ = 1.6 Hz), 4.69 (s, 4H, $H_{17a,b}$) 4.44 – 4.33 (m, 4H, $H_{10a,b}$), 4.15 - 4.11 (m, 1H, D_2), 3.99 (dd, 1H, H_2, $J_{2\text{-}1}$ = 1.6 Hz, $J_{2\text{-}3}$ = 3.1 Hz), 3.96 – 3.91 (m, 1H, H_{6a}), 3.90 - 3.73 (m, 5H, D_1, $H_{6b,}$ $H_{7a,b}$, $H_{3,}$), 3.73 - 3.62 (m, 2H, H_4, H_5), 3.53 – 3.41 (m, 2H, $H_{8a,b}$), 3.08 - 2.93 (m, 2H, D_4,D_5), 2.13 - 1.96 (m, 4H, D_3, D_6).

^{13}C NMR (100 MHz, CD_3OD): δ = 177.3, 177.1 (C_9); 162.0 (d, C_{13}, $J_{13\text{-}F}$ = 245.0 Hz); 142.1 (d, C_{11}, $J_{11\text{-}F}$ = 7.3 Hz); 130.6 (d, C_{15}, $J_{15\text{-}F}$ = 5.0 Hz); 128.4, 128.3 (d, C_{14}, $J_{14\text{-}F}$ = 15.2 Hz); 124.2, 124.1 (d, C_{16}, $J_{16\text{-}F}$ = 4.7 Hz); 115.1, 115.0 (d, C_{12}, $J_{13\text{-}F}$ = 22.5 Hz); 100.5 (C_1); 76.6 (C_3); 75.7 (C_5); 72.8 (C_{D1}); 72.6 (C_2); 72.5 (D_2); 69.3 (C_7); 69.0 (C_4); 63.2 (C_6); 58.8 (d, C_{17}, $J_{17\text{-}F}$ = 4.3 Hz); 52.2 (C_8); 43.4 (C_{10}); 42.1, 42.0 (C_{D4}, C_{D5}); 29.9, 29.0 (C_{D3}, C_{D6}).

2.4.8.8 N^1,N^2-bis(4,5-di-(hydroxymethylene)benzyl)amide, 2.2q

1,2-Cyclohexanedicarboxamides-N^1,N^2-bis(4,5-(dhydroxymethylene)benzyl)-4-(2-azidoethoxy)-5-[α-D-mannopyranosyloxy]- (1*S*,2*S*,4*S*,5*S*)

Prepared according to general procedure 5, using amine **2.12q.**



2.2q

Yield = 63 %;

$[\alpha]_D^{20}$ = + 9.4 (c = 0.2 in methanol)

MS (HRMS) calculated for: $[C_{34}H_{47}N_5O_{13}Na]^+$: 756.30681; found: 756.30576.

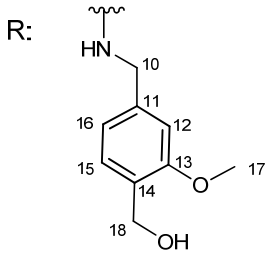
^{1}H NMR (400 MHz, CD_3OD): δ = 7.20 (s, 2H, H_{14}), 7.13 (s, 2H, H_{12}), 4.95 (br s, 1H, H_1), 4.67 - 4.46 (m, 8H, H_{15}), 4.36 - 4.24 (m, 4H, H_{10}), 4.01 (d, 1H, H_5, J = 2.7 Hz), 3.90 (dd, 1H, H_2, $J_{2\text{-}1}$ = 1.7 Hz, $J_{2\text{-}3}$ = 3.2 Hz), 3.88 (d, 1H, H_{6b}, $J_{6\text{-}6}$ = 11.3 Hz), 3.83 - 3.76(m, 1H, H_3), 3.76 - 3.63 (m, 4H, D_2, H_{6a}, H_7), 3.62 - 3.52 (m, 2H, H_4, D_1), 3.45 - 3.33 (m, 2H, H_8), 3.04 - 2.84 (m, 2H, D_4, D_5), 2.07 - 1.90 (m, 4H, D_3, D_6).

^{13}C NMR (100 MHz, CD_3OD): δ = 177.9, 177,7 (C_9); 143.8, (C_{13}); 140.9 (C_{11}); 126.4, 126.3 (C_{12}); 125.8 (C_{14}); 100.0 (C_1); 77.2 (C_3); 76.2 (C_{D1}); 73.2 (C_{D2}); 73.1 (C_5); 72.9 (C_2); 69.8 (C_7); 69.4 (C_4); 65.2(C_{15}); 63.6 (C_6); 52.2 (C_8); 44.4 (C_{10}); 42.5, 42.3 (C_{D4}, C_{D5}); 30.5, 29.6 (C_{D3}, C_{D6}).

2.4.8.9 N^1,N^2-bis(3-methoxy-4-(hydroxymethylene)benzyl)amide, 2.2r

1,2-Cyclohexanedicarboxamides-N^1,N^2-bis(3-methoxy-4-(hydroxymethylene)benzyl)-4-(2-azidoethoxy)-5-[α-D-mannopyranosyloxy]- (1*S*,2*S*,4*S*,5*S*)

Prepared according to general procedure 5, using amine **2.12r.**



2.2r

Yield = 80 %

$[\alpha]_D^{20}$ = - 24.3 (c = 0.15 in methanol)

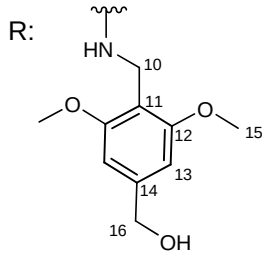
MS (HRMS) calculated for: $[C_{34}H_{47}N_5O_{13}Na]^+$: 756.30681; found: 756.30567

^{1}H NMR (400 MHz, CD_3OD): δ = 7.23 (d, 2H, H_{15}, $J_{15\text{-}16}$ = 7.6 Hz), 6.81 (s, 2H, H_{12}), 6.80 (d, 2H, H_{16}, $J_{15\text{-}16}$ = 7.6 Hz), 4.94 (br s, 1H, H_1), 4.55 (s, 4H, H_{18}), 4.27 (m, 4H, H_{10}), 4.04 (d, 1H, H_5, $J_{6\text{-}5}$ = 2.7 Hz), 3.91 (dd, 1H, H_2, $J_{2\text{-}1}$ = 1.7 Hz, $J_{2\text{-}3}$ = 3.2 Hz), 3.87 - 3.81 (m, 1H, H_{6b}), 3.79 - 3.76 (m, 6H, H_{17}), 3.76 - 3.72 (m, 2H, H_3, D_2), 3.72. - 3.64 (m, 3H, H_{6a}, H_7), 3.59 - 3.54 (m, 2H, H_4, D_1), 3.44 - 3.33 (m, 2H, H_8), 3.08 - 2.77 (m, 4H, D_4, D_5), 2.05 - 1.82 (m, 4H, D_3, D_6).
^{13}C NMR (100 MHz, CD_3OD): δ = 177.3, 177.0 (C_9); 158.6 (C_{13}); 140.9, 140.8 (C_{11}); 129.5 (C_{14}); 129.1 (C_{15}); 120.2, 120.1 (C_{16}); 110.4, 110.3 (C_{12}); 100.4 (C_1); 76.7 (C_3); 75.7 (C_{D1}); 72.7 (C_{D2}); 72.5 (C_5); 72.4 (C_2); 69.3 (C_7); 68.9 (C_4); 63.2 (C_6); 60.4 (C_{18}); 56.0 (C_{17}); 52.1 (C_8); 43.9 (C_{10}); 42.0, 41.9 (C_{D4}, C_{D5}); 30.0, 29.1 (C_{D3}, C_{D6}).

2.4.8.10 N^1,N^2-bis(2,6-dimethoxy-4-(hydroxymethylene)benzyl)amide, 2.2s

1,2-Cyclohexanedicarboxamides-N^1,N^2-bis(2,6-dimethoxy-4-(hydroxymethylene)benzyl)-4-(2-azidoethoxy)-5-[α-D-mannopyranosyloxy]- (1*S*,2*S*,4*S*,5*S*)

Prepared according to general procedure 5, using amine **2.12s.**



2.2s

Yield = 28 %

$[\alpha]_D^{20}$ = + 36.5 (c = 0.1 in methanol)

MS (HRMS) calculated for: $[C_{36}H_{51}N_5O_{15}Na]^+$: 816.32794; found: 816.32600

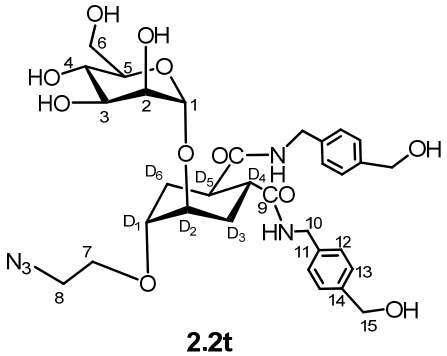
^{1}H NMR (400 MHz, CD_3OD): δ = 6.63 (s, 4H, H_{13}), 4.97 (s, 1H, H_1), 4.63 (s, 4H, H_{16}), 4.46 (dd, 2H, H_{10a}, $J_{10a\text{-}NH}$ = 7.6 Hz, $J_{10a\text{-}10b}$ = 13.4 Hz), 4.17 (dd, 2H, H_{10b}, $J_{10b\text{-}10a}$ = 5.8 Hz), 3.97 (d, 1H, H_5, $J_{6\text{-}5}$ = 2.6 Hz), 3.90 - 3.76 (m, 14H, $H_{15'}$, H_2, H_{6b}), 3.74 - 3.59 (m, 5H, H_7, H_{6a}, H_3, D_2), 3.59 - 3.48 (m, 2H, H_4, D_1), 3.35 - 3.32 (m, 4H, H_8), 2.81 - 2.62 (m, 2H, D_4, D_5), 1.98 - 1.72 (m, 4H, D_3, D_6). **^{13}C NMR** (100 MHz, CD_3OD): δ = 176.4, 176.1 (C_9); 160.2, 160.1 (C_{12}); 144.7 (C_{11}); 129.1 (C_{14}); 103.3 (C_{13}); 100.4 (C_1); 76.8 (C_3); 75.6 (C_{D1}); 72.7 (C_{D2}); 72.5 (C_5); 72.2 (C_2); 69.3

(C_7); 68.9 (C_4); 65.4 (C_{16}); 63.2 (C_6); 56.4 (C_{15}); 52.1 (C_8); 42.1, 42.0 (C_{D4}, C_{D5}); 33.5, 33.3 (C_{10}); 29.5, 28.6 (C_{D3}, C_{D6}).

2.4.8.11 1,2-Cyclohexanedicarboxamides-*N*^{1},*N*^{2}-bis(4-hydroxymethylenebenzyl)-4-(2-azidoethoxy)-5-[α-D-mannopyranosyloxy]- (1*R*,2*R*,4*R*,5*R*), 2.2t (diastereoisomer of 2.2f)

1,2-Cyclohexanedicarboxamides-*N*^{1},*N*^{2}-bis(4-hydroxymethylenebenzyl)-4-(2-azidoethoxy)-5-[α-D-mannopyranosyloxy]- (1*R*,2*R*,4*R*,5*R*)

Starting from a 4:1 mixture of diastereoisomers **2.31** and **2.43**, and amine **2.12f** Prepared according to general procedure 5. The separation of the isomers were obtained after the first step, before the deprotection, (silica, Et_2O with gradient of EA from 50% to 100%, TLC (Et_2O/EA = 1:1): Rf (1*S*, 2*S*, 3*S*, 4*S*) = 0.16, Rf (1*R*, 2*R*, 3*R*, 4*R*) = 0.2)



2.2t

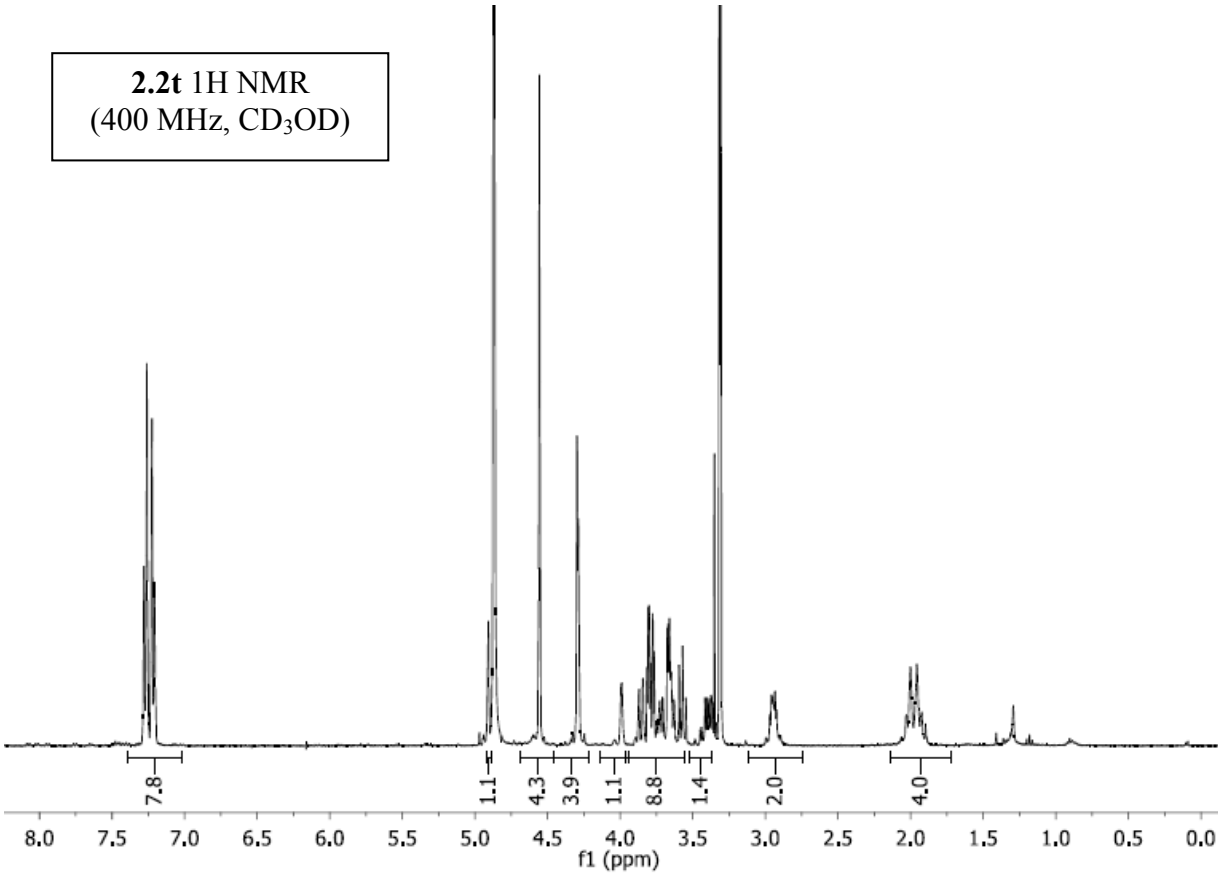
[α]D20 = + 36.9 (c = 0.23 in methanol)

MS (HRMS) calculated for: $[C_{32}H_{43}N_5O_{11}Na]^+$ = 696.8568, found: 696.8560

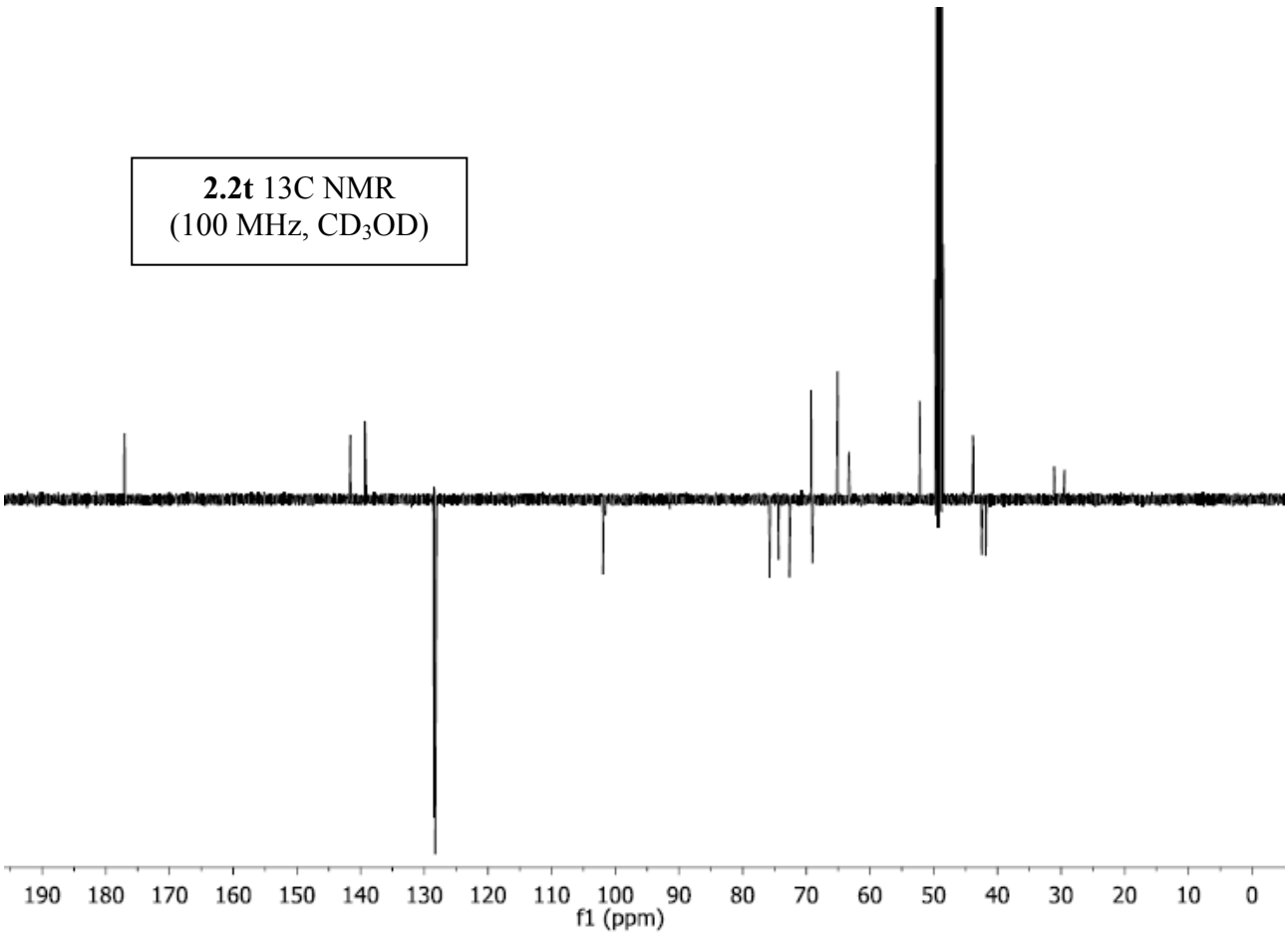
^{1}H NMR (400 MHz, CD_3OD): 7.27 (d, 4H, H_{12}, $J_{12\text{-}13}$ = 8.0 Hz), 7.22 (d, 4H, H_{13}, $J_{13\text{-}12}$ = 8.0 Hz), 4.91 (d, 1H, H_1, $J_{1\text{-}2}$ = 1,5 Hz), 4.56 (s, 4H, $H_{15a,b}$) 4.30 – 4.27 (m, 4H, $H_{10a,b}$), 4.01 – 3.97 (m, 1H, D_2), 3.88 – 3.82 (m, 1H, H_{6a}), 3.82 – 3.74 (m, 3H, H_2, $H_{7a,b}$,), 3.74 - 3.69 (m, 1H, H_3), 3.69 - 3.61 (m, 3H, D_1, H_5, H_{6b}), .3.60 – 3.54 (m, 1H, H_4), 3.45 – 3.33 (m, 2H, $H_{8a,b}$), 3.01 - 2.84 (m, 2H, D_4,D_5), 2.07 - 1.88 (m, 4H, D_3, D_6).

^{13}C NMR (100 MHz, CD_3OD): 177.2, 177.0 (C9); 141.7, 141.6 (C_{14}); 139.3, 139.2 (C_{11}); 128.5 128.5 (C_{12}); 128.3, 128.3 (C_{13}); 101.9 (C_1); 75.8, 75.7 (C_3, C_5); 74.4 (C_{D2}); 72.7 (C_2); 72.4 (D_1); 69.2 (C_7); 69.0 (C_4); 65.1, 65.1 (C_{15}); 63.3 (C_6); 52.2 (C_8); 43.8, 43.7 (C_{10}); 42.5, 41.8 (C_{D4}, C_{D5}); 31.1, 29.5 (C_{D3}, C_{D6}).

2.2t 1H NMR
(400 MHz, CD_3OD)

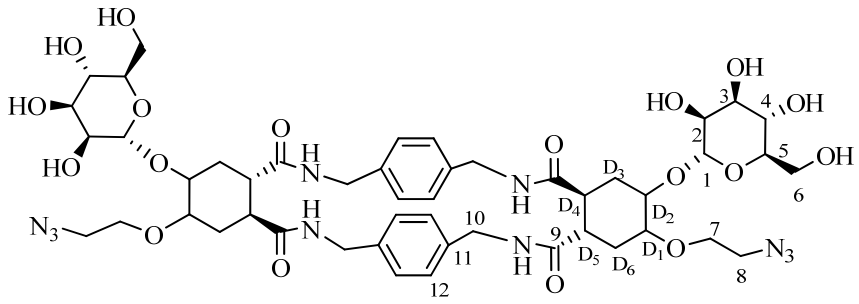


2.2t 13C NMR
(100 MHz, CD_3OD)



2.4.8.12 Macrocycle, 2.47

To the flask charged with scaffold **2.31** (60 mg, 0.055 mmoli, 1 eq) a solution of p-xylilbenzylamine **2.45a** (3.81 mg, 0.028 mmol, 0.5 eq) in 0.55 mL of MeCN was added under nitrogen. The reaction was stirred for 5 h. TLC (silica, DCM:MeOH = 9:1, DCM:MeOH = 9:1+1% TEA, Hex:AcOEt = 6:4) indicated no presence of amine **2.45a** but scaffold **2.31** was still present, therefore another portion of amine **2.45a** (0.5 eq) was added and the reaction mixture was stirred for additional 16 h. TLC indicated again no presence of amine **2.45a** but still presence of scaffold **2.12**, therefore another portion of amine **2.45a** (0.3 eq) was added and the reaction mixture was stirred for additional 16 h. The solvent was removed under reduced pressure and the crude was purified by flash chromatography (silica, hexane with gradient of EA from 30 % to 70 %) to afford 18.6 mg of intermediate with macrocyclic structure. **MS (ESI)** calculated for $[C_{104}H_{98}N_{10}O_{26}Na]^+$: 1926,9; found: 1926.6. To the solution of the product obtained in the previous reaction (18.6 mg, 0.0097 mmol, 0.18 eq) in dry methanol (0.7 ml), a solution of sodium methoxide in MeOH (1M, 100 µL, 0.037 mmoli, 4 eq) was added. After 45 min the reaction mixture was diluted with methanol and neutralized with prewashed Amberlite IRA 120-H^+. The resin was filtered off and the filtrate was concentrated under reduced pressure. The crude was purified by flash chromatography ($CHCl_3$ with gradient of methanol from 0 to 20% with 10% water in methanol) to afford 8.7 mg of product.



2.47

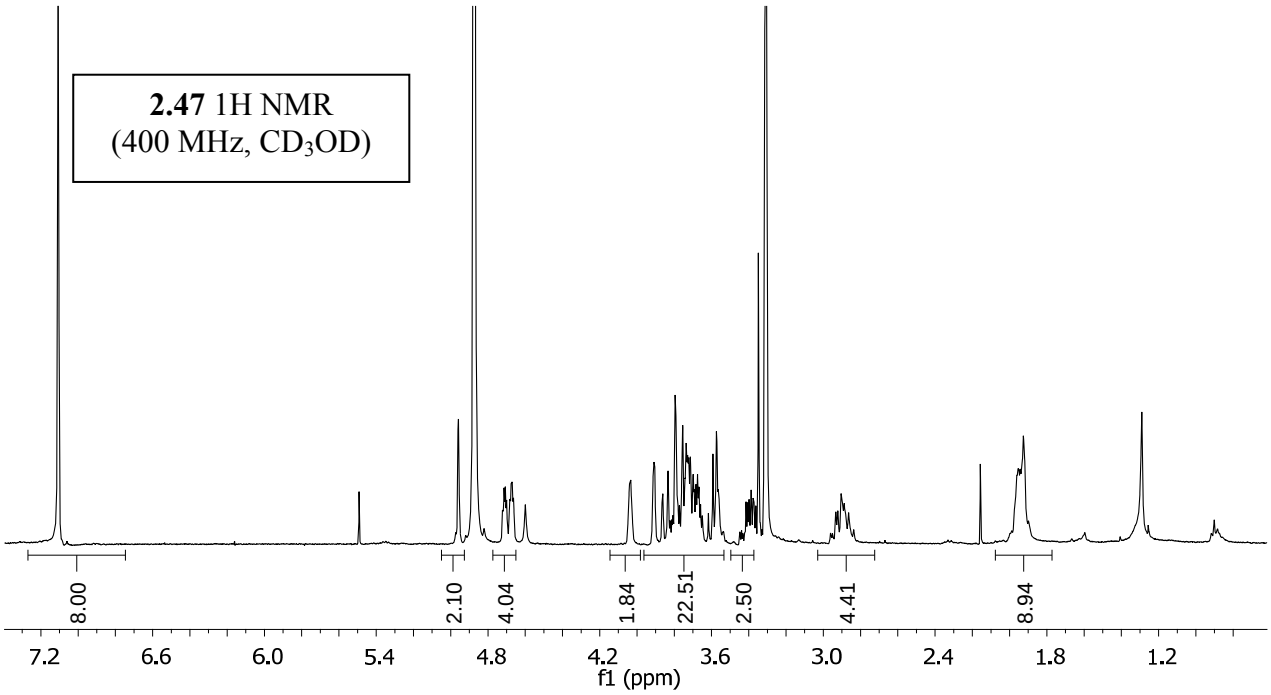
only one isomer shown

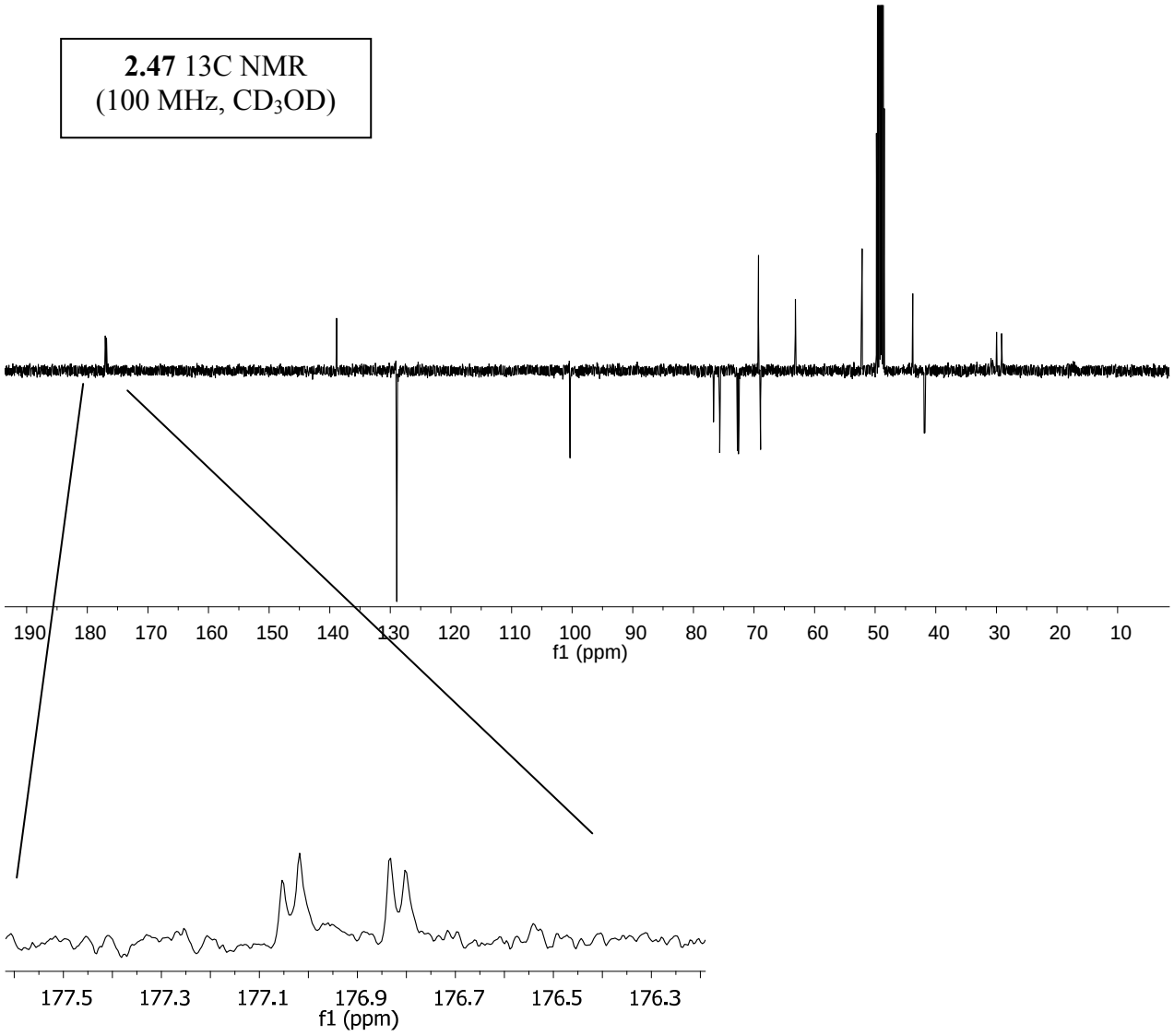
Yield = 15 %, **Note:** The macrocyle was isolated as a 1:1 mixture of diastereoisomers, as shown by the ^{13}C spectrum.

MS (HRMS) calculated for: $[C_{48}H_{66}N_{10}O_{18}Na]^+$: 1093,44543; found: 1093.44341.

^{1}H NMR (400 MHz, CD_3OD): δ = 7.11 (s, 8H, H_{12}), 4.96 (d, 2H, H_1, $J_{2\text{-}1}$ = 1.7 Hz), 4.72 – 4.65 (m, 4H, H_{10a}), 4.05 – 4.02 (m, 2H, D_2), 3.91 (dd, 2H, H_2, $J_{2\text{-}1}$ = 1.7 Hz, $J_{2\text{-}3}$ = 3.2 Hz), 3.88 - 3.81 (m, 2H, H_{6b}), 3.80 - 3.62 (m, 14H, H_{10b}, H_3, H_7, D_1, H_{6b}), 3.61 - 3.52 (m, 2H, H_4, H_5), 3.45 - 3.33 (m, 2H, H_8), 2.98 - 2.81 (m, 2H, D_4, D_5), 2.00 - 1.86 (m, 4H, D_3, D_6).

^{13}C NMR (100 MHz, CD_3OD): δ = 177.1, 177.0, 176.8, 176.7 (C_9); 138.9, 138.9 (C_{11}); 128.9 (C_{12}); 100.3 (C_1); 76.7 (C_{D1}); 75.7 (C_5); 72.7 (C_3); 72.6 (C_2); 72.5 (C_{D2}); 69.3 (C_7); 68.9 (C_4); 63.2 (C_6); 52.2 (C_8); 43.8 (C_{10}); 41.9, 41.8 (C_{D4}, C_{D5}); 30.0, 29.1 (C_{D3}, C_{D6}).





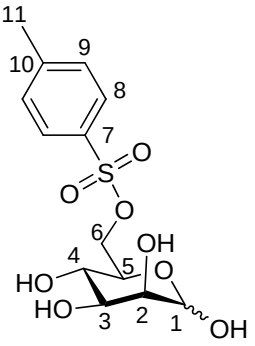
2.4.9 Synthesis and characterization of 48a-e, 49b, 53-61

2.4.9.1 6-azido-D-6-deoxymannopyranose, 2.53

6-(p-toluensulfonyl)-D-6-deoxymannopyranose

To the suspension of mannose 2.52 (0.5 g, 2.77 mmol, 1 eq) in 7 ml of pyridine a solution of TsCl (1g, 5.55 mmol, 2 eq) in 3 ml of pyridine was added at room temperature. The reaction was stirred at room temperature for 3 h but the TLC (DCM:MeOH = 8:2) indicated still some starting material so, and additional portion of TsCl (0.5 g, 2.75 mmol, 1 eq) was added and the reaction was stirred overnight. The pyridine was removed under reduced pressure and the crude residue was dissolved in a mixture of DCM:MeOH (9:1) and filtered through a pad of sodium carbonate in order to remove the excess of TsCl, TsOH and scavenge the HCl. The filtrate was

concentrated under reduced pressure and the crude residue was purified by silica ($CHCl_3$ with gradient of MeOH from 0 to 15%) to obtain 2.6 g of 6-(p-toluensulfonyl)-D-6-deoxymannopyranose as mixture of α and β anomers in 4:1 ratio.



Yield: 50%.

MS (ESI) calculated for $[C_{13}H_{18}O_8SNa]^+$: 357.3; found: 357.2

^{1}H NMR (400 MHz, CD_3OD):

α anomer : 7.80 (d, 2H, H_8, $J_{8\text{-}9}$ =8.3 Hz), 7.43 (d, 2H, H_9, $J_{8\text{-}9}$ =8.3 Hz), 5.00 (d, 1H, H_1, $J_{1\text{-}2}$ = 1.4 Hz), 4.39 - 4.23 (m, 1H, H_{6A}), 4.19 – 4.07 (m, 1H, H_{6B}), 3.93 - 3.82 (m, 1H, H_5), 3.75 (dd, 1H, H_2, $J_{1\text{-}2}$ =1.4 Hz, $J_{3\text{-}2}$ =3.3 Hz), 3.70 (dd, 1H, H_3, $J_{3\text{-}4}$ = 9.3 Hz, $J_{3\text{-}2}$ =3.3 Hz), 3.51 (t, 1H, H_4, $J_{3\text{-}4}$ = 9.3), 2.45 (s, 1H, H_{11}).

β anomer : 7.80 (d, 2H, H_8, $J_{8\text{-}9}$ =8.3 Hz), 7.43 (d, 2H, H_9, $J_{8\text{-}9}$ =8.3 Hz), 4.69 (d, 1H, H_1, $J_{1\text{-}2}$ = 0.8 Hz), 4.39 - 4.23 (m, 1H, H_{6A}), 4.19 – 4.07 (m, 1H, H_{6B}), 3.93 - 3.82 (m, 1H, H_5), 3.78 – 3.76 (m, 1H, H_2), 3.70 (dd, 1H, H_3, $J_{3\text{-}4}$ = 9.3 Hz, $J_{3\text{-}2}$ =3.3 Hz), 3.51 (t, 1H, H_4, $J_{3\text{-}4}$ = 9.3), 2.45 (s, 1H, H_{11}).

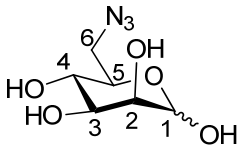
^{13}C NMR (100 MHz, CD_3OD, the assignment of the NMR shifts for the α and β anomers was obtained using the HSQC spectrum**):**

α anomer : 146.5 (C_7); 134.7 (C_{10}); 131.1 (C_9); 129.3 (C_8); 96.0 (C_1); 72.9 (C_2); 72.3 (C_3); 71.8 (C_5); 71.5 (C_6); 68.5 (C_4); 21.7 (C_{11}).

β anomer : 146.5 (C_7); 134.7 (C_{10}); 131.2 (C_9); 129.3 (C_8); 95.8 (C_1); 75.6 (C_2); 75.3 (C_3); 73.1 (C_5); 71.3 (C_6); 68.2 (C_4); 21.7 (C_{11}).

The 6-tosylate obtained in the previous reaction (2.5 g, 7.4 mmol, 1 eq) was dissolved in DMF (15 ml) and to this solution TBAI (catalytic amount) and NaN_3 (1.5 g, 22 mmol, 3eq) were added. The solution was stirred at 60°C for 3 days. Then the solvent was removed under reduced

pressure and the crude was purified by a short pad of silica (EA with gradient of MeOH from 0 to 20%) to obtain 1.35 g of product as a mixture of α and β anomers in 3.7:1 ratio.



2.53

Yield: 89%.

MS (ESI) calculated for $[C_6H_{11}N_3O_5Na]^+$: 228.2; found: 228.1

^{1}H NMR (400 MHz, CD_3OD):

α anomer : 5.24 (br s, 1H, H_1), 4.42 (dd, 1H, H_5, $J_{5\text{-}6}$ =0.9 Hz, $J_{5\text{-}4}$ = 5.8 Hz), 4.08 (dd, 1H, H_{6A}, $J_{6A\text{-}6B}$ = 7.1 Hz, $J_{6A\text{-}5}$ = 0.9 Hz), 3.83 (dd, 1H, H_2, $J_{1\text{-}2}$ =1.5 Hz, $J_{3\text{-}2}$ = 5.4 Hz), 3.77 (br s, 1H, H_3), 3.65 (t, 1H, H_4, $J_{3\text{-}4}$ = 6.4), 3.67 - 3.61 (m, 1H, H_{6B}).

β anomer : 5.08 (d, 1H, H_1, $J_{1\text{-}2}$ = 0.8 Hz), 4.42 (dd, 1H, H_5, $J_{5\text{-}6}$ =0.9 Hz, $J_{5\text{-}4}$ = 5.8 Hz), 4.08 (dd, 1H, H_{6A}, $J_{6A\text{-}6B}$ = 7.1 Hz, $J_{6A\text{-}5}$ = 0.9 Hz), 3.79 (dd, 1H, H_2, $J_{1\text{-}2}$ = 0.8 Hz, $J_{3\text{-}2}$ = 7.1 Hz), 3.77 (br s, 1H, H_3), 3.67 - 3.61 (m, 1H, H_{6B}), 3.58 (t, 1H, H_4, $J_{3\text{-}4}$ = 6.4).

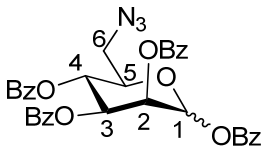
^{13}C NMR (100 MHz, CD_3OD, the assignment of the NMR shifts for the α and β anomers was obtained using the HSQC spectrum**):**

α anomer : 103.5 (C_1); 77.5 (C_5); 73.3 (C_3); 72.5 (C_2); 67.8 (C_4); 65.9 (C_6).

β anomer : 96.0 (C_1); 77.5 (C_5); 73.0 (C_3); 72.2 (C_2); 69.8 (C_4); 65.9 (C_6).

2.4.9.2 (1,2,3,4-*O*-tetrabenzoyl)-6-azido-D-6-deoxymannopyranose, 2.54

To the solution of 6-azido-mannose **2.53** (1.3 g, 6.34 mmol, 1 eq) in pyridine (30 ml) BzCl (5.9 ml, 5.72 mmol, 6 eq) was added at 0°C. The reaction was stirred at room temperature for 1 h then heated up to 70°C for 3h. The reaction was let to cool to room temperature then the solvent was removed under reduced pressure. The crude was taken up in ether and washed with 1M HCl and water. The organic layer was dried over sodium sulphate and concentrated under reduced pressure. The crude product was purified by flash chromatography (silica, hexane with gradient of EA from 5 % to 20 %) to afford 1.97 g of product as a mixture of α and β anomers in 4:1 ratio.



2.54

Yield: 50%

MS (ESI) calculated for $[C_{34}H_{27}N_3O_9Na]^+$: 644.6; found: 644.3.

^{1}H NMR (400 MHz, $CDCl_3$):

α anomer: δ = 8.20 – 7.20 (m, 20H, H_{Bz}), 6.36 (d, 1H, H_1, $J_{1\text{-}2}$ = 1.1 Hz), 6.07 (dd, 1H, H_2, $J_{1\text{-}2}$ = 1.1 Hz, $J_{3\text{-}2}$ = 3.2 Hz), 5.94 (t, 1H, H_4, $J_{4\text{-}3}$ = $J_{4\text{-}5}$= 9.8 Hz), 5.75 (dd, 1H, H_3, $J_{3\text{-}4}$ = 9.8 Hz, $J_{3\text{-}2}$= 3.2), 4.20 – 4.13 (m, 1H, H_5), 3.61 (dd, 1H, H_{6a}, $J_{5\text{-}6a}$ = 2.8 Hz, $J_{6a\text{-}6b}$= 13.5 Hz), 3.53 (dd, 1H, H_{6b}, $J_{5\text{-}6a}$ = 5.5 Hz, $J_{6a\text{-}6b}$= 13.5 Hz).

β anomer: δ = 8.20 – 7.20 (m, 20H, H_{Bz}), 6.60 (d, 1H, H_1, $J_{1\text{-}2}$ = 2.0 Hz), 6.05 – 6.00 (m, 1H, H_4),), 5.88 – 5.85 (m, 1H, H_2),), 5.74 – 5.63 (m, 1H, H_3), 4.41 – 4.34 (m, 1H, H_5),), 3.55 – 3.41 (m, 2H, H_{6a}, H_{6b}),

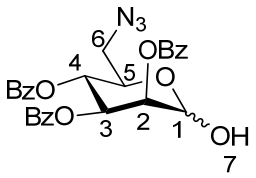
^{13}C NMR (100 MHz, $CDCl_3$, the assignments of the NMR shifts for the α and β anomers were obtained using the HSQC spectrum):

α anomer: δ = 165.9, 165.6, 165.6, 164.3 (CO_{BZ}); 134.0, 133.9, 133.8, 133.7 (CH_{BZ}); 130.4, 130.3, 130.0 (CH_{BZ}); 129.5 ($Cquat_{BZ}$); 128.9, 128.8 (CH_{BZ}); 128.8 ($Cquat_{BZ}$); 128.7, 128.6 (CH_{BZ}); 91.3 (C_1); 74.9 (C_5); 71.5 (C_3); 69.5 (C_2); 67.3 (C_4); 51.1 (C_6).

β anomer: δ = 165.9, 165.6, 165.6, 164.3 (CO_{BZ}); 134.3, 133.9, 133.8, (CH_{BZ}); 130.8, 130.4, 130.3, 130.0 (CH_{BZ}); 129.5 ($Cquat_{BZ}$); 129.1, 129.0 (CH_{BZ}); 128.8 ($Cquat_{BZ}$); 128.8, 128.6 (CH_{BZ}); 91.4 (C_1); 74.9 (C_5); 69.5 (C_4); 68.6 (C_3); 67.2 (C_2); 51.1 (C_6).

2.4.9.3 (2,3,4-*O*-tribenzoyl)-6-azido-D-6-deoxymannopyranosyl trichloroacetoimidate, 2.49b

To a solution of mannose derivative **2.54** (120 mg, 0.193 mmol, 1 eq) in dry THF (0.5 ml) a methylamine solution in ethanol (33%, 0.074 ml, 0.57 mmol, 3 eq) was added at 0°C under nitrogen. The reaction was stirred at 0°C for 1 h. The solvent was removed under reduced pressure and the crude was purified by flash chromatography (silica, hexane with gradient of EA from 5 % to 30 %) to afford 84 mg of product as mainly the α anomer.



Yield: 84%

MS (ESI) calculated for $[C_{27}H_{23}N_3O_8Na]^+$: 540.5; found: 540.0.

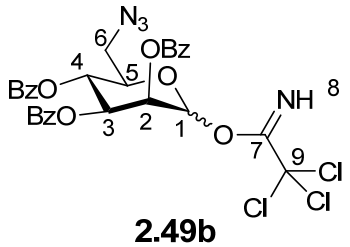
^{1}H NMR (400 MHz, $CDCl_3$):

<u>α anomer</u>: δ = 8.13 – 8.04 (m, 2H, H_{Bz}), 8.00 – 7.90 (m, 2H, H_{Bz}), 7.87 – 7.75 (m, 2H, H_{Bz}), 7.67 – 7.56 (m, 1H, H_{Bz}), 7.55 – 7.45 (m, 3H, H_{Bz}), 7.43 – 7.32 (m, 3H, H_{Bz}), 7.29 – 7.18 (m, 2H, H_{Bz}), 5.96 (dd, 1H, H_3, $J_{3\text{-}4}$ = 10.0 Hz, $J_{3\text{-}2}$= 3.3), 5.87 (t, 1H, H_4, $J_{4\text{-}3}$ = $J_{4\text{-}5}$= 10.0 Hz), 5.71 (dd, 1H, H_2, $J_{1\text{-}2}$ = 1.6 Hz, $J_{3\text{-}2}$ = 3.3 Hz), 5.52 (d, 1H, H_1, $J_{1\text{-}2}$ = 1.6 Hz), 4.54 – 4.43 (m, 1H, H_5), 3.51 – 3.45 (m, 2H, $H_{6a,b}$), 3.35 (br s, 1H, H_7).

^{13}C NMR (100 MHz, $CDCl_3$):

<u>α anomer</u>: δ = 165.8, 165.8, 165.7 (CO_{BZ}); 133.8, 133.8, 133.4 (CH_{BZ}); 130.2, 130.0, 130.0 (CH_{BZ}); 129.5, 129.3, 129.0 ($Cquat_{BZ}$); 128.9, 128.7, 128,5 (CH_{BZ}); 92.5 (C_1); 70.9 (C_2); 70.4 (C_5); 69.6 (C_3); 68.0 (C_4); 51.6 (C_6).

To the solution of mannose derivative obtained in the previous reaction (72 mg, 0.139 mmol, 1 eq) in dry DCM (0.5 ml) trichloroacetonitrile (0.07 ml, 0.696 mmol, 5 eq) and a catalytic amount of DBU (cca 0.01 ml) was added at room temperature under nitrogen. The reaction was stirred at room temperature for 1 h. The solvent was removed under reduced pressure and the crude was purified by flash chromatography (silica, hexane with gradient of EA from 5 % to 30 %) to afford 73 mg of product as mainly the α anomer.



2.49b

Yield: 80%

MS (ESI) calculated for $[C_{29}H_{23}C_{l3}N_4O_8Na]^+$: 684.9; found: 685.1

^{1}H NMR (400 MHz, $CDCl_3$):

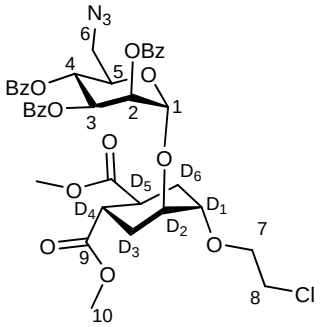
α anomer: δ = 8.88 (s, 1H, H_8), 8.13 – 8.08 (m, 2H, H_{Bz}), 7.97 – 7.93 (m, 2H, H_{Bz}), 7.83 – 7.78 (m, 2H, H_{Bz}), 7.65 – 7.59 (m, 1H, H_{Bz}), 7.55 – 7.46 (m, 3H, H_{Bz}), 7.45 – 7.33 (m, 3H, H_{Bz}), 7.29 – 7.22 (m, 2H, H_{Bz}), 5.56 (d, 1H, H_1, $J_{1\text{-}2}$ = 1.8 Hz), 5.99 (t, 1H, H_4, $J_{4\text{-}3}$ = $J_{4\text{-}5}$= 9.8 Hz), 5.95 – 5.86 (m, 2H, H_2, H_3), 4.48 – 4.41 (m, 1H, H_5), 3.54 (dd, 1H, H_{6a}, $J_{5\text{-}6a}$ = 2.8 Hz, $J_{6a\text{-}6b}$= 13.6 Hz), 3.49 (dd, 1H, H_{6b}, $J_{5\text{-}6a}$ = 5.4 Hz, $J_{6a\text{-}6b}$= 13.6 Hz).

^{13}C NMR (100 MHz, $CDCl_3$):

α anomer: δ = 165.4, 165.2 (CO_{BZ}); 159.8 (C_7); 133.8, 133.7 133.4 (CH_{BZ}); 130.0, 129.9, 129.7 (CH_{BZ}); 129.9, 128.8 ($Cquat_{BZ}$); 128.7 (CH_{BZ}); 128.6 ($Cquat_{BZ}$); 128.6, 128.4 (CH_{BZ}); 100.0 (C_9); 94.5 (C_1); 72.9 (C_5); 69.5, 68.7 (C_2, C_3); 66.9 (C_4); 50.9 (C_6).

2.4.9.4 1,2-Cyclohexanedicarboxylic acid, 4-(2-chloroethoxy)-5-((2,3,4-*O*-tribenzoyl)-6-azido-α-D-6-deoxymannopyranosyloxy)-, 1,2-dimethyl ester, (1*S*,2*S*,4*S*,5*S*) 2.51b

A mixture of the acceptor **2.50**[8] (25.5 mg, 0.086 mmol, 1 eq.) and the donor **2.49b** (70 mg, 0.105 mmol, 1.22 eq.) was coevaporated with toluene three times. Powdered and activated acid washed 4Å molecular sieves were added; the mixture was kept under vacuum for a few h and then dissolved with dry CH_2Cl_2 (1 mL) under nitrogen. After cooling at −30°C, TMSOTf (4 µL, 0.017 mmol, 0.2 eq.) was added to the reaction mixture under stirring. The reaction was stirred at -20°C for 1 h. The reaction was quenched with Et_3N and the mixture warmed to room temperature and filtered over a celite pad. The filtrate was evaporated at reduced pressure and the crude product purified by flash chromatography (hexane with gradient of ethyl acetate from 5 % to 40 %) to yield 61 % of pure product**.**



2.51b

Yield: 80 %

MS (ESI) calculated for $[C_{39}H_{40}ClN_3NaO_{13}]^+$: 817.2; found 816.5

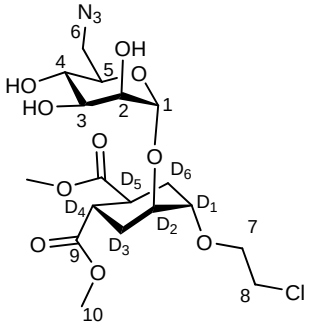
^{1}HNMR (400 MHz, $CDCl_3$): 8.1 – 8.05 (m, 2H, H_{Bz}), 7.99 – 7.89 (m, 2H, H_{Bz}), 7.83 – 7.74 (m, 2H, H_{Bz}), 7.65 – 7.56 (m, 1H, H_{Bz}), 7.55 – 7.46 (m, 3H, H_{Bz}), 7.45 – 7.33 (m, 3H, H_{Bz}), 7.29 – 7.17 (m, 2H, H_{Bz}), 5.84 – 5.76 (m, 2H, H_3, H_4), 5.66 (dd, 1H, H_2, $J_{2\text{-}1}$ = 1.9 Hz, $J_{2\text{-}3}$ = 2.7 Hz),

5.24 (d, 1H, H_1, $J_{1\text{-}2}$ = 1.6 Hz), 4.29 (t, 1H, H_5, $J_{6a\text{-}5} = J_{5\text{-}4}$ = 7.2 Hz), 4.06 (dd, 1H, D_2, $J_{D1\text{-}D2}$ = 4.0 Hz, $J_{D2\text{-}3 \text{ or } 6}$ = 7.8 Hz), 3.93 – 3.83 (m, 1H, H_{7a}), 3.81 – 3.67 (m, 8H, H_{7b}, H_2, H_{10}), 3.63 (t, 2H, H_8, $J_{8\text{-}7}$ = 5.77 Hz), 3.57 (dd, 1H, H_{6a}, $J_{6b\text{-}5}$ = 7.2 Hz, $J_{6a\text{-}6b}$ = 13.3 Hz), 3.40 (dd, 1H, H_{6a}, $J_{6b\text{-}5}$ = 2.3 Hz, $J_{6a\text{-}6b}$ = 13.3 Hz), 3.13 - 3.00 (m, 2H, D_4, D_5), 2.17 – 1.97 (m, 4H, D_3, D_6).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.9, 174.8 (C_9); 165.9, 165.7, 165.6 (CO_{BZ}); 133.9, 133.5, 133.6, 133.5 (CH_{BZ}); 130.1, 130.1, 129.9, (CH_{BZ}); 129.4, 129.1 ($Cquat_{BZ}$); 128.9 (CH_{BZ}); 128.8 ($Cquat_{BZ}$); 128.7, 128.5 (CH_{BZ}); 96.3 (C_1); 75.5 (C_{D1}); 73.3 (C_{D2}); 71.3 (C_5); 70.9 (C_2); 69.7 (C_7); 69.7 (C_3); 68.0 (C_4); 52.3, 52.3 (C_{10}); 51.6 (C_6); 43.3 (C_8); 39.4, 39.3 (C_{D4},C_{D5}); 28.2 (C_{D3}); 27.5 (C_{D6}).

2.4.9.5 1,2-Cyclohexanedicarboxylic acid, 4-(2-chloroethoxy)-5-(6-azido-α-D-6-deoxymannopyranosyloxy)-, 1,2-dimethyl ester, (1*S*,2*S*,4*S*,5*S*) 2.55

Compound **2.51b** (435 mg, 0.548 mmol, 1 eq) was dissolved in dry methanol (5 ml), under nitrogen at room temperature, and a solution of sodium methoxide in MeOH (1 M, 1 ml, 1 mmol, 2 eq) was added. After reaction completion (1 h, TLC: DCM:MeOH = 9:1) the reaction mixture was diluted with methanol and neutralized with prewashed Amberlite IRA 120-H^+. The resin was filtered off and the filtrate was concentrated under reduced pressure. The crude was purified by flash chromatography (silica, $CHCl_3$ with gradient of methanol from 3% to 20%) to afford 248 mg of product.



2.55

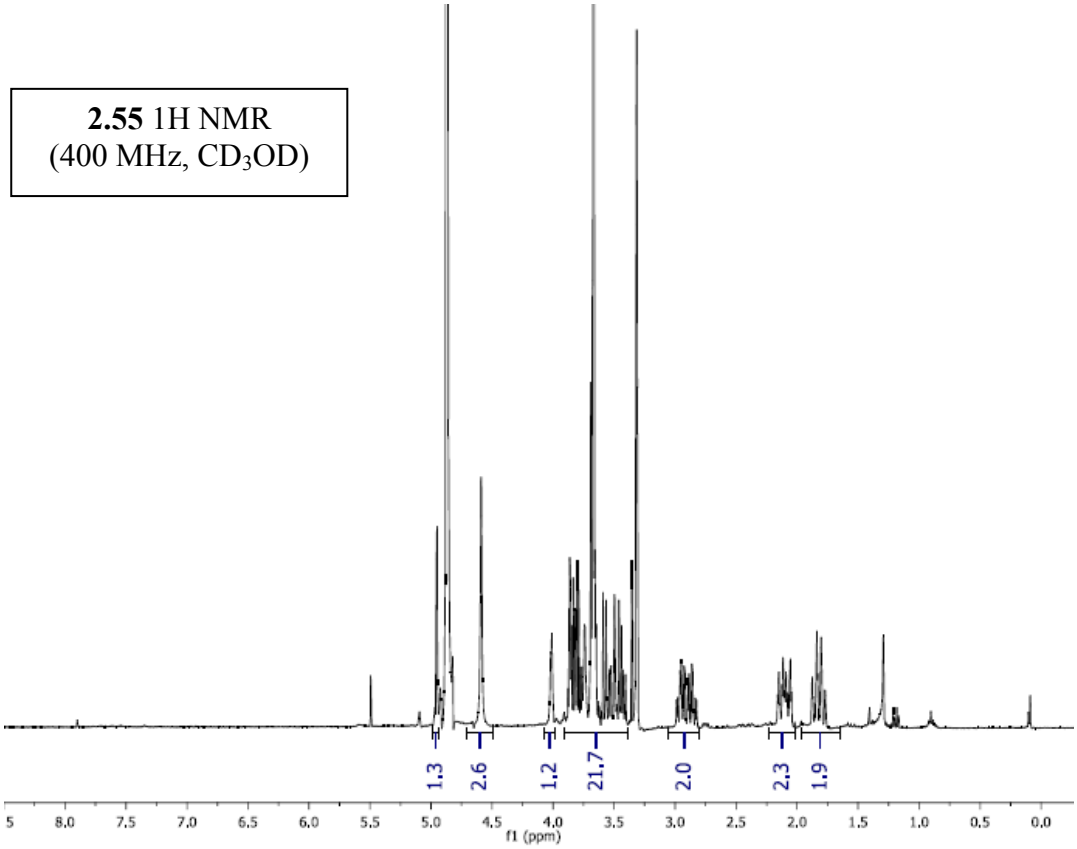
Yield = 94 %

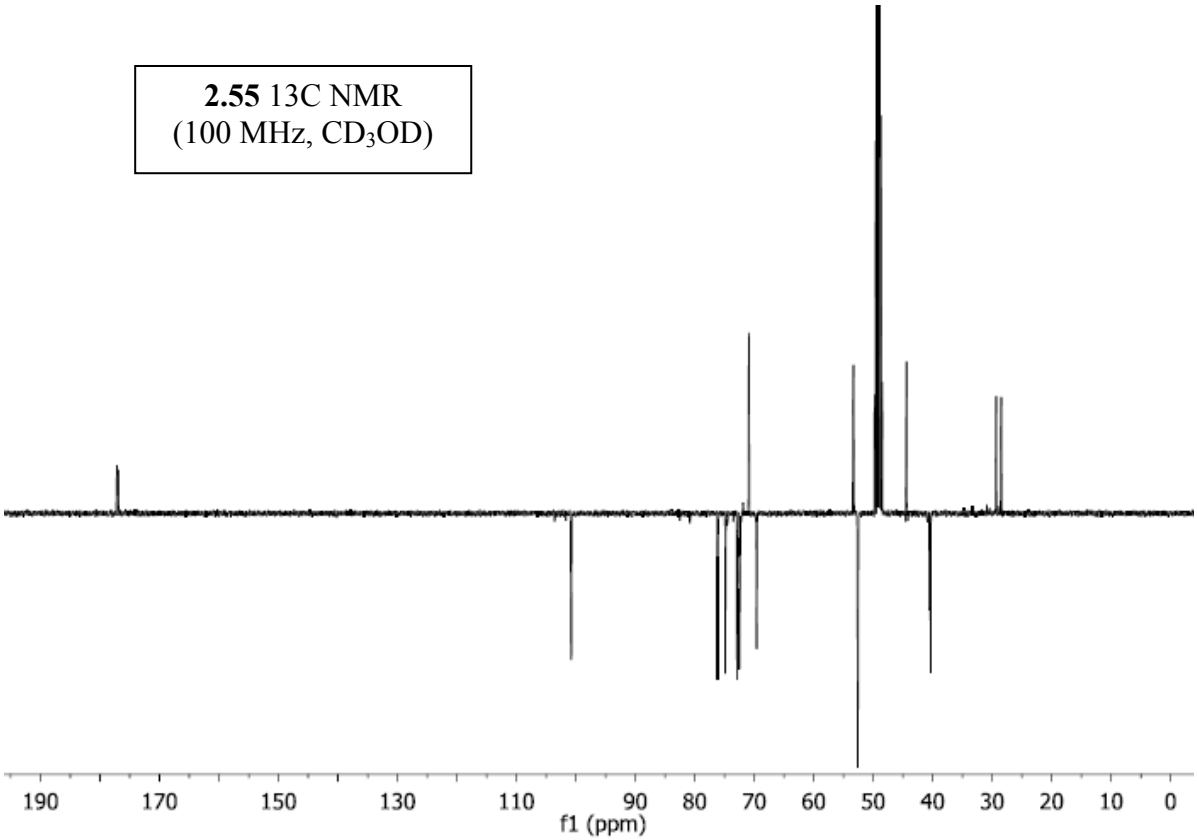
MS (ESI) calculated for: $[C_{18}H_{28}ClN_3O_{10}Na]^+$: 504.9; found: 504.3

^{1}H NMR (400 MHz, CD_3OD): δ = 4.95 (d, 1H, H_1, $J_{1\text{-}2}$ = 1.6 Hz), 4.03 – 3.99 (m, 1H, D_2), 3.94 – 3.89 (m, 1H, H_{6a}), 3.88 – 3.76 (m, 4H, H_2, H_3, H_7), 3.75 – 3.71 (m, 1H, D_1), 3.71 - 3.64 (m, 9H, H_8, H_5, H_{10}), 3.61 – 3.54 (m, 1H, H_4), 3.51 (dd, 1H, H_{6a}, $J_{6a\text{-}6b}$ = 13.0 Hz, $J_{6a\text{-}5}$ = 2.1 Hz), 3.43 (dd, 1H, H_{6a}, $J_{6a\text{-}6b}$ = 13.0 Hz, $J_{6a\text{-}5}$ = 8.1 Hz), 3.00 – 2.81 (m, 2H, D_4,D_5), 2.21 - 1.94 (m, 2H, D_{3ax}, D_{6ax}), 1.94 – 1.72 (m, 2H, D_{3eq}, D_{6eq})

^{13}C NMR (100 MHz, CD_3OD): δ = 177.2, 176.9 (C_9); 100.8 (C_1); 76.2 (C_{D1}); 74.9 (C_5); 72.9 (D_2); 72.5, 72.4 (C_2, C_3); 70.9 (C_7); 69.6 (C_4); 53.3 (C_6); 52.6 (C_{10}); 44.4 (C_8); 40.5, 40.4 (C_{D4}, C_{D5}); 29.4, 28.5 (C_{D3}, C_{D6}).

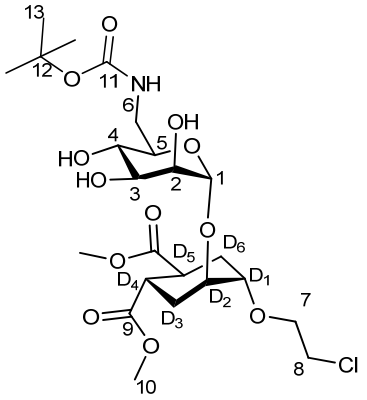
2.55 1H NMR
(400 MHz, CD_3OD)





2.4.9.6 1,2-Cyclohexanedicarboxylic acid, 4-(2-chloroethoxy)-5-(6-N-carbo-tbutoxy-α-D-6-deoxymannopyranosyloxy)-, 1,2-dimethyl ester, (1*S*,2*S*,4*S*,5*S*) 2.56

To a solution of **2.55** (50 mg, 0.103 mmol, 1 eq) and Boc_2O (33 mg, 0.154 mmol, 1.5 eq) in methanol (10 ml) 10 % Pd/C was added in catalytic amount. The reaction was stirred under H_2 (1 atm) at room temperature for 2 h. The catalyst was filtered off through a celite pad. The filtrate was concentrated under reduced pressure to yield 53 mg of pure product.



2.56

Yield = 94 %

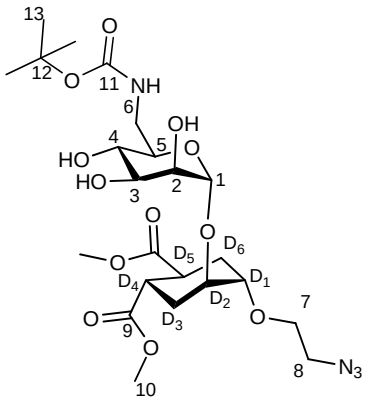
MS (ESI) calculated for: $[C_{23}H_{38}ClNO_{12}Na]^+$: 579.0; found: 578.3

^{1}H NMR (400 MHz, CD_3OD): δ = 4.90 (d, 1H, H_1, $J_{1\text{-}2}$ = 1.6 Hz), 4.98 – 3.93 (m, 1H, D_2), 3.85 – 3.74 (m, 3H, H_2, H_7), 3.72 – 3.62 (m, 10H, H_{10}, D_1, H_8, H_3), 3.62 – 3.45 (m, 3H, H_4, H_5, H_{6a}), 3.19 (dd, 1H, H_{6b}, $J_{6a\text{-}6b}$ = 13.3 Hz, $J_{6a\text{-}5}$ = 5.1 Hz), 2.97 – 2.78 (m, 2H, D_4, D_5), 2.14 - 1.98 (m, 2H, D_{3ax}, D_{6ax}), 1.86 – 1.71 (m, 2H, D_{3eq}, D_{6eq}).

^{13}C NMR (100 MHz, CD_3OD): δ = 177.1, 177.0 (C_9); 158.7 (C_{11}); 101.1 (C_1); 80.4 (C_{12}); 76.1 (C_{D1}); 74.3 (C_5); 73.2 (D_2); 72.6 (C_2); 72.4 (C_3); 70.9 (C_7); 69.9 (C_4); 52.5 (C_{10}); 44.5 (C_8); 42.9 (C_6); 40.5, 40.3 (C_{D4}, C_{D5}); 29.4 (C_{D3} or C_{D6}); 29.0 (C_{13}); 28.5 (C_{D3} or C_{D6}).

2.4.9.7 1,2-Cyclohexanedicarboxylic acid, 4-(2-azidoethoxy)-5-(6-amino-α-D-6-deoxymannopyranosyloxy)-, 1,2-dimethyl ester, (1*S*,2*S*,4*S*,5*S*), 2.48a

To a solution of **2.56** (50 mg, 0.0899 mmol, 1 eq.) in DMF (1 mL) sodium azide (35 mg, 0.539 mmol, 5 eq.) was added. The reaction was stirred at 50°C for 4 days. The solvent was removed at reduced pressure and the crude residue was purified by flash chromatography (silica, chloroform with gradient of methanol from 3% to 20%) to afford 48 mg of product.



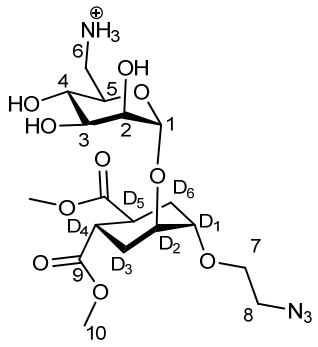
Yield = 94 %

MS (ESI) calculated for: $[C_{23}H_{38}N_4O_{12}Na]^+$: 585.6; found: 585.3

^{1}H NMR (400 MHz, CD_3OD): δ = 4.84 (d, 1H, H_1, $J_{1\text{-}2}$ = 1.6 Hz), 3.93 – 3.89 (m, 1H, D_2), 3.78 – 3.77 (m, 1H, H_2), 3.74 – 3.64 (m, 2H, H_7), 3.65 – 3.55 (m, 8H, H_{10}, D_1, H_3), 3.51 – 3.36 (m, 3H, H_4, H_5, H_{6a}), 3.31 (t, 2H, H_8, $J_{7\text{-}8}$ = 4.7 Hz), 3.12 (dd, 1H, H_{6b}, $J_{6a\text{-}6b}$ = 13.5 Hz, $J_{6a\text{-}5}$ = 5.4 Hz), 2.90 – 2.72 (m, 2H, D_4, D_5), 2.08 - 1.92 (m, 2H, D_{3ax}, D_{6ax}), 1.80 – 1.63 (m, 2H, D_{3eq}, D_{6eq})

^{13}C NMR (100 MHz, CD_3OD): δ = 177.1, 176.9 (C_9); 158.7 (C_{11}); 101.1 (C_1); 80.4 (C_{12}); 76.2 (C_{D1}); 74.4 (C_5); 73.1 (D_2); 72.6 (C_2); 72.4 (C_3); 69.9 (C_4); 69.7 (C_7); 52.5 (C_{10}); 52.2 (C_8); 42.8 (C_6); 40.5, 40.2 (C_{D4}, C_{D5}); 29.2 (C_{D3} or C_{D6}); 29.0 (C_{13}); 28.6 (C_{D3} or C_{D6}).

The product obtained in previous reaction (45 mg, 0.080 mmol, 1 eq.) was dissolved in TFA (1 ml). The resulting solution was stirred at 35°C for 3 h. The solvent was removed under reduced pressure and the crude residue was washed twice with a small amount diethyl ether. The product was dried under reduced pressure to afford 32 mg of product.



2.48a

Yield = 87 %;

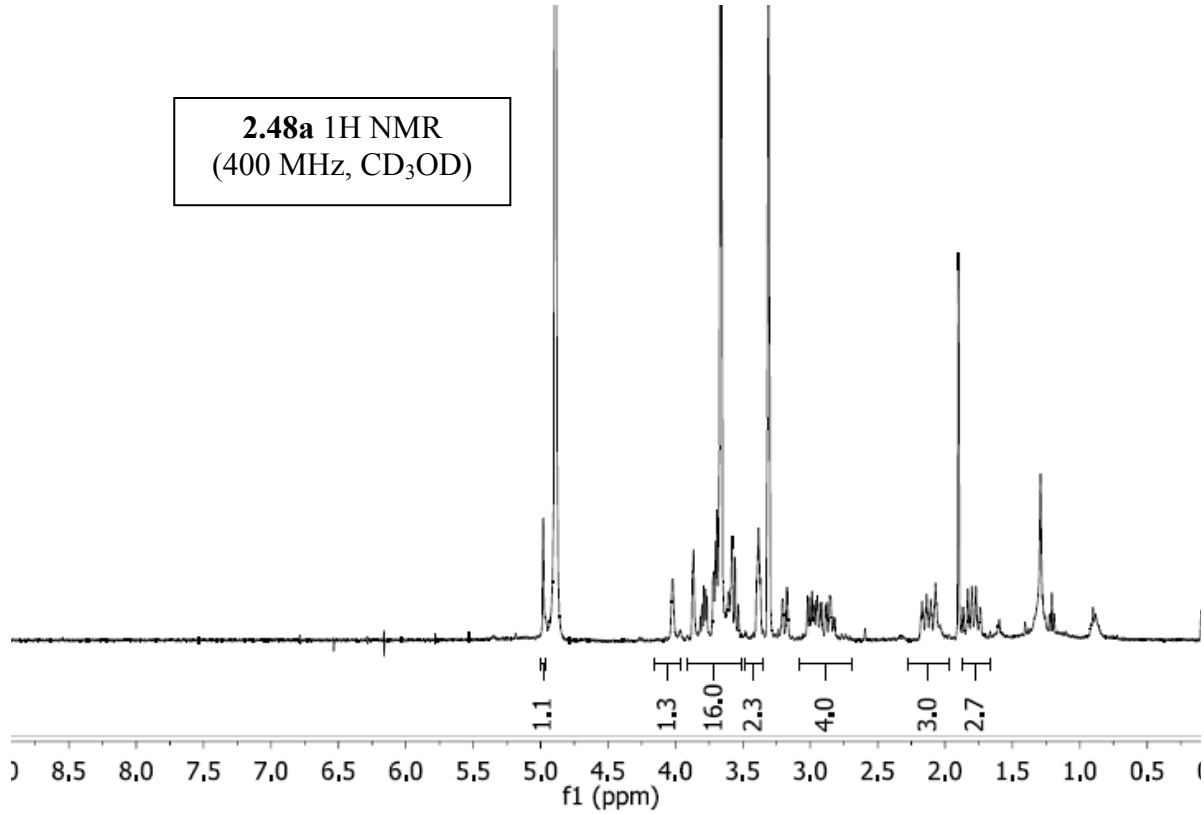
$[\alpha]_D^{20}$ = + 19.2 (c = 0.2 in methanol)

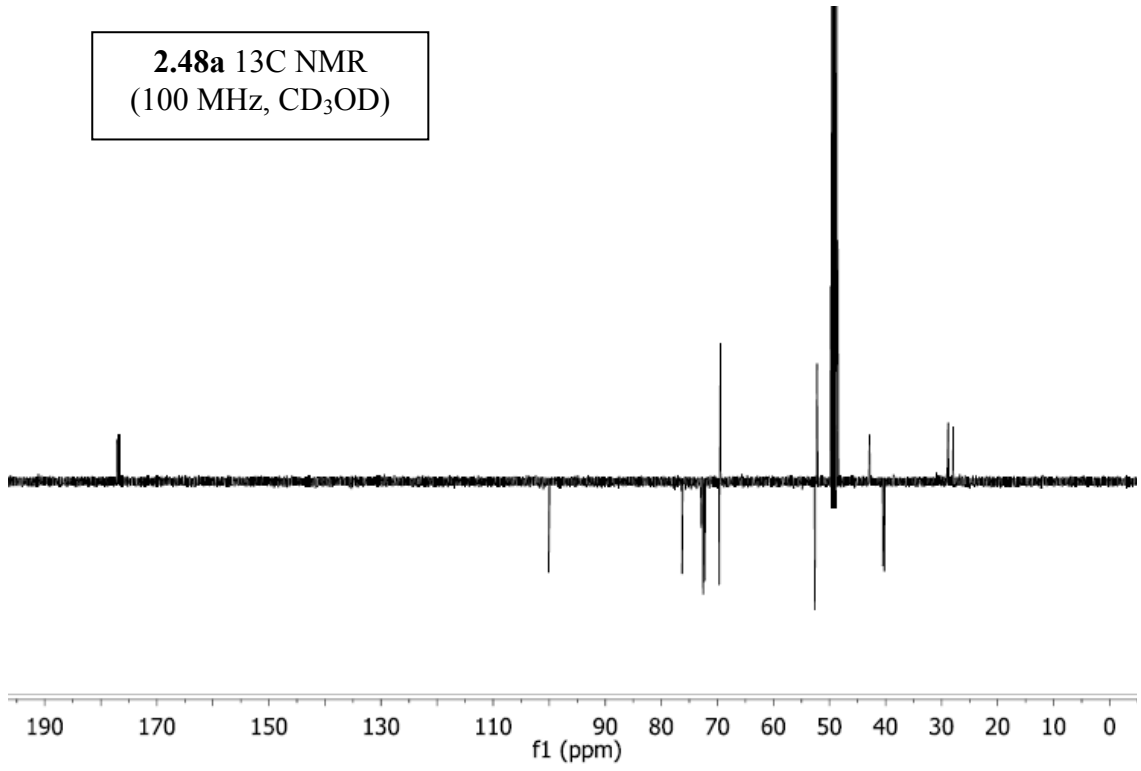
MS (HRMS) calculated for: $[C_{18}H_{30}N_4O_{10}Na]^+$: 485.18596; found: 485.18621

$[C_{18}H_{31}N_4O_{10}]^+$: 463.20347; found: 463.20399

^{1}H NMR (400 MHz, CD_3OD): δ = 4.98 (d, 1H, H_1, $J_{1\text{-}2}$ = 1.6 Hz), 4.04 – 3.89 (m, 1H, D_2), 3.87 (dd, 1H, H_2, $J_{1\text{-}2}$ = 1.6 Hz, $J_{3\text{-}2}$ = 3.0 Hz), 3.82 – 3.76 (m, 1H, H_{7a}), 3.74 – 3.63 (m, 9H, H_{10}, D_1, H_3, H_{7b}), 3.63 – 3.50 (m, 2H, H_4, H_5), 3.42 – 3.34 (m, 2H, H_8), 3.19 (dd, 1H, H_{6a}, $J_{6a\text{-}6b}$ = 13.2 Hz, $J_{6a\text{-}5}$ = 2.6 Hz), 3.04 – 2.79 (m, 3H, H_{6b}, D_4, D_5), 2.20 – 2.00 (m, 2H, D_{3ax}, D_{6ax}), 1.88 – 1.70 (m, 2H, D_{3eq}, D_{6eq})

^{13}C NMR (100 MHz, CD_3OD): δ = 177.1, 176.8 (C_9); 100.1 (C_1); 76.3 (C_{D1}); 72.9 (C_5); 72.5 (C_2); 72.4 (D_2); 72.2 (C_3); 69.7 (C_4); 69.4 (C_7); 52.6, 52.6 (C_{10}); 52.2 (C_8); 42.8 (C_6); 40.5, 40.2 (C_{D4}, C_{D5}); 28.8 , 28.9(C_{D3}, C_{D6}).

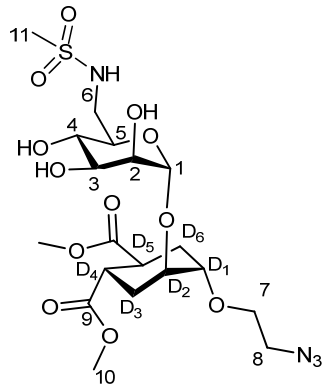




2.4.9.8 1,2-Cyclohexanedicarboxylic acid, 4-(2-azidoethoxy)-5-(6-N-methansulfonyl-α-D-6-deoxymannopyranosyloxy)-1,2-dimethyl ester, (1*S*,2*S*,4*S*,5*S*), 2.48b

To a solution of **2.48a** (15 mg, 0.0324 mmol, 1 eq.) in acetonitrile (0.5 mL) MsCl (3.2 µl, 0.042 mmol, 1.3 eq.) and DIPEA (8.3 µl, 0.0648 mmol, 2 eq) were added under nitrogen. The reaction

was stirred at room temperature for 1 h. The solvent was removed at reduced pressure and the crude residue was purified by flash chromatography (silica, chloroform with gradient of methanol from 2% to 20%) to afford 5.5 mg of product.



2.48b

Yield = 45 %;

$[\alpha]_D^{20}$ = + 28.2 (c = 0.11 in methanol)

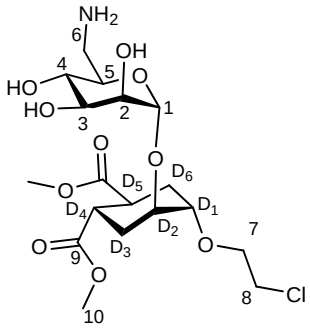
MS (HRMS) calculated for: $[C_{19}H_{32}N_4O_{12}SNa]^+$: 563,16351; found: 563.16366

^{1}H NMR (400 MHz, CD_3OD): δ = 4.93 (s, 1H, H_1), 4.09 – 4.02 (m, 1H, D_2), 3.87 – 3.82 (m, 1H, H_2), 3.81 – 3.73 (m, 3H, D_1, H_7), 3.71 – 3.62 (m, 7H, H_{10}, H_3), 3.62 – 3.45 (m, 3H, H_4, H_5, H_{6a}), 3.38 – 3.33 (m, 2H, H_8), 3.14 (dd, 1H, H_{6b}, $J_{6a\text{-}6b}$ = 13.5 Hz, $J_{6a\text{-}5}$ = 5.8 Hz), 3.03 – 2.77 (m, 5H, H_{11}, D_4, D_5), 2.20 – 2.02 (m, 2H, D_{3ax}, D_{6ax}), 1.87 – 1.69 (m, 2H, D_{3eq}, D_{6eq})

^{13}C NMR (100 MHz, CD_3OD): δ = 177.2, 176.9 (C_9); 100.6 (C_1); 76.0 (C_{D1}); 74.6 (C_5); 72.6 (C_2); 72.4 (C_3); 72.3 (D_2); 69.8 (C_4); 69.8 (C_7); 52.6 (C_{10}); 52.3 (C_8); 45.5 (C_6); 40.5, 40.2, 40.1 (C_{11}, C_{D4}, C_{D5}); 29.3, 28.3 (C_{D3}, C_{D6})

2.4.9.9 1,2-Cyclohexanedicarboxylic acid, 4-(2-chloroethoxy)-5-(6-amino-α-D-6-deoxymannopiranosyloxy)-1,2-dimethyl ester, (1*S*,2*S*,4*S*,5*S*), 2.57

To a solution of **2.55** (17 mg, 0.0353 mmol, 1 eq) in methanol (7 ml) 10 % Pd/C was added in catalytic amount. The reaction was stirred under H_2 (1 atm) at room temperature for 2 h. The catalyst was filtered off through a celite pad. The filtrate was concentrated under reduced pressure to yield 17 mg of product with some impurities, no further purification was performed.



2.57

Yield = (96%)

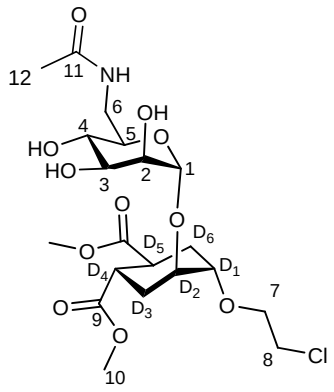
MS (ESI) calculated for: $[C_{18}H_{30}ClNO_{10}Na]^+$: 455.9; found: 456.3

^{1}H NMR (400 MHz, CD_3OD): δ = 4.99 (s, 1H, H_1), 4.05 – 3.94 (m, 1H, D_2,), 3.91 – 3.85 (m, 12H, , H_2, H_{10}, D_1, H_3, H_7, H_5), 3.59 – 3.42 (m, 2H, H_4, H_8), 3.35 – 3.23 (m, 1H, H_{6a}), 3.10 (dd, 1H, H_{6b}, $J_{6a\text{-}6b}$ = 13.1 Hz, $J_{6a\text{-}5}$ = 7.7 Hz), 3.14 – 3.06 (m, 2H,, D_4, D_5), 2.15 – 1.96 (m, 2H, D_{3ax}, D_{6ax}), 1.88 – 1.70 (m, 2H, D_{3eq}, D_{6eq}).

^{13}C NMR (100 MHz, CD_3OD): δ = 177.0, 176.7 (C_9); 100.0 (C_1); 76.1 (C_{D1}); 72.7, 72.5, 72.0, 71.2 (D_2, C_2, C_3, C_5); 70.7 (C_7); 69.6 (C_4); 52.6 (C_{10}); 44.6 (C_8); 42.2 (C_6); 40.4, 40.3 (C_{D4}, C_{D5}); 29.0, 28.0 (C_{D3}, C_{D6}).

2.4.9.10 1,2-Cyclohexanedicarboxylic acid, 4-(2-azidoethoxy)-5-(6-N-acetyl-α-D-6-deoxymannopyranosyloxy)-1,2-dimethyl ester, (1*S*,2*S*,4*S*,5*S*), 2.48c

To a solution of **2.57** (10 mg, 0.02 mmol, 1 eq) in MeCN (1 ml) AcCl (5 µl, 0.06 mmol, 3 eq) and DIPEA (10 µl, 0.06 mmol, 3 eq) was added at room temperature under nitrogen. The reaction was stirred at room temperature for 3 h. TLC (DCM:MeOH = 9:1 and 8:1) indicated no starting material but several products. Probably the free hydroxyl groups got acetylated. The solvent was removed under reduced pressure and the crude residue was dissolved in dry MeOH (1 ml) under nitrogen. In order to deprotect the acetylated hydroxyl groups, sodium methoxide (0.5 M, 0.18 ml, 0.06 mol, 3 eq) was added. The reaction was stirred overnight. The reaction mixture was diluted with methanol and neutralized with prewashed Amberlite IRA 120-H^+. The resin was filtered off and the filtrate was concentrated under reduced pressure. The crude was purified by flash chromatography (silica, $CHCl_3$ with gradient of methanol from 3% to 20%) to afford 5.9 mg of product.

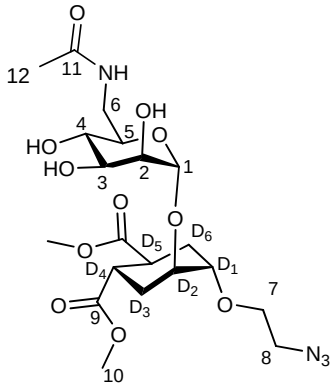


Yield = 60 %

MS (ESI) calculated for: $[C_{20}H_{32}ClNO_{11}Na]^+$: 520.9; found: 520.5

^{1}H NMR (400 MHz, CD_3OD): δ = 4.92 (d, 1H, H_1, $J_{1\text{-}2}$ = 1.4 Hz), 3.98 – 3.93 (m, 1H, D_2), 3.87 – 3.83 (m, 1H, H_2), 3.83 – 3.78 (m, 1H, H_{7a}), 3.78 – 3.71 (m, 1H, H_{7b}), 3.71 – 3.61 (m, 11H, H_{10}, D_1, H_8, H_3, H_{6a}), 3.60 – 3.41 (m, 2H, H_4, H_5), 3.36 – 3.31 (m, 1H, H_{6b}), 2.99 – 2.79 (m, 2H, D_4, D_5), 2.13 – 2.01 (m, 2H, D_{3ax}, D_{6ax}), 1.97 (s, 3H, H_{12}), 1.88 – 1.71 (m, 2H, D_{3eq}, D_{6eq})
^{13}C NMR (100 MHz, CD_3OD): δ = 177.1, 176.9 (C_9); 173.7 (C_{11}); 100.3 (C_1); 76.2 (C_{D1}); 73.6 (C_5); 72.6 (C_2); 72.3, 72.3 (C_3, D_2); 70.8 (C_7); 69.8 (C_4); 52.6 (C_{10}); 44.5 (C_8); 41.8 (C_6); 40.5, 40.3 (C_{D4}, C_{D5}); 29.2, 28.2 (C_{D3}, C_{D6}); 22.8 (C_{12}).

To a solution of the compound obtained in the previous reaction (5 mg, 0.01 mmol, 1 eq) in DMF (0.1 ml) sodium azide (3.5 mg, 0.05 mmol, 5 eq.) was added. The reaction was stirred at 50°C for 4 days. The solvent was removed at reduced pressure and the crude residue was purified by flash chromatography (silica, chloroform with gradient of methanol from 3% to 20%) to afford 5 mg of product.



2.48c

Yield = quant.

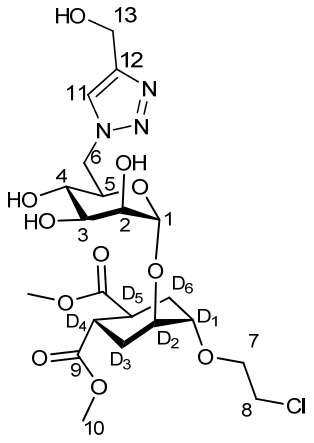
$[\alpha]_D^{20}$ = + 34.4 (c = 0.125 in methanol)

MS (HRMS) calculated for: $[C_{20}H_{32}N_4O_{11}Na]^+$: 527.19653; found: 527.19650

^{1}H NMR (400 MHz, CD_3OD): δ = 4.93 (d, 1H, H_1, $J_{1\text{-}2}$ = 1.6 Hz), 4.02 – 3.94 (m, 1H, D_2), 3.85 (dd, 1H, H_2, $J_{1\text{-}2}$ = 1.6 Hz, $J_{3\text{-}2}$ = 3.3 Hz), 3.80 – 3.73 (m, 1H, H_{7a}), 3.71 – 3.61 (m, 10H, H_{10}, D_1, H_{7b}, H_3, H_{6a}), 3.60 – 3.45 (m, 2H, H_4, H_5), 3.42 – 3.34 (m, 3H, H_8, H_{6b}), 3.04 – 2.75 (m, 2H, D_4, D_5), 2.18 – 2.03 (m, 2H, D_{3ax}, D_{6ax}), 1.97 (s, 3H, H_{12}), 1.87 – 1.67 (m, 2H, D_{3eq}, D_{6eq}).
^{13}C NMR (100 MHz, CD_3OD): δ = 177.1, 176.9 (C_9); 173.7 (C_{11}); 100.4 (C_1); 76.4 (C_{D1}); 73.5 (C_5); 72.6 (C_2); 72.3, 72.3 (C_3, D_2); 69.8 (C_4); 69.6 (C_7); 52.6 (C_{10}); 52.2 (C_8); 41.8 (C_6); 40.5, 40.2 (C_{D4}, C_{D5}); 28.9, 28.1 (C_{D3}, C_{D6}); 22.7 (C_{12}).

2.4.9.11 1,2-Cyclohexanedicarboxylic acid, 4-(2-chloroethoxy)-5-(6-((4-hydroxymethylene)triazol-1-yl)-α-D-6-deoxymannopyranosyloxy)-1,2-dimethyl ester, (1*S*,2*S*,4*S*,5*S*), 2.58

To a solution of propargyl alcohol (4.7 mg, 0.083 mmol, 2 eq) in THF (0.5 ml) and water (0.5 ml) TBTA (8.8 mg, 0.0166 mmol, 0.4 eq.), copper(II) sulphate (0.5 mg, 0.002 mmol, 0.05 eq.) and sodium ascorbate (3.3 mg, 0.0166 mmol, 0.4 eq.) were added. The reaction was stirred at room temperature for 10 minutes then **2.55** was added (20 mg, 0.0415 mmol, 1 eq.). The reaction was stirred overnight at room temperature under nitrogen. The solvent was removed at reduced pressure and the crude residue was purified by flash chromatography (silica, chloroform with gradient of methanol from 3% to 30%) to afford 13.7 mg of product.



2.58

Yield = 61 %

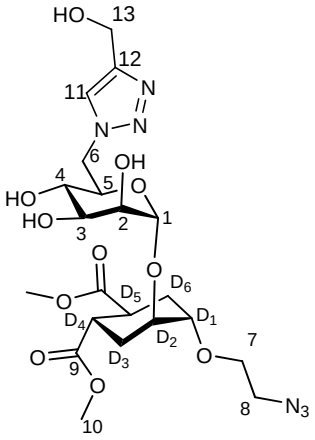
MS (HRMS) calculated for: $[C_{21}H_{32}ClN_3O_{11}Na]^+$: 560.9; found: 560.4

^{1}H NMR (400 MHz, CD_3OD): δ = 7.92 (s, 1H, H_{11}), 4.91 – 4.81 (m, 2H, H_1, H_{6a}), 4.67 (s, 2H, H_{13}), 4.44 (dd, 1H, H_{6b}, $J_{6a\text{-}6b}$ = 14.2 Hz, $J_{6b\text{-}5}$ = 9.3 Hz), 3.87 (dd, 1H, H_2, $J_{1\text{-}2}$ = 1.8 Hz, $J_{2\text{-}3}$ = 9.5 Hz), 3.83 (dd, 1H, H_5, $J_{5\text{-}4}$ = 3.0 Hz, $J_{5\text{-}6a}$ = 1.8 Hz), 3.71 (dd, 1H, H_3, $J_{3\text{-}4}$= 3.0 Hz, $J_{5\text{-}3}$ = 9.4 Hz), 3.67 – 3.50 (m, 11H, H_{10}, D_2, H_{7a}, H_4, H_8), 3.50 – 3.42 (m, 1H, H_{7b}), 3.16 – 3.11 (m, 1H, D_1), 2.89 – 2.72 (m, 2H, D_4, D_5), 1.98 – 1.88 (m, 2H, D_{3ax}, D_{6ax}), 1.75 – 1.55 (m, 2H, D_{3eq}, D_{6eq}).

^{13}C NMR (100 MHz, CD_3OD): δ = 177.1, 176.8 (C_9); 149.0 (C_{12}); 125.8 (C_{11}); 100.9 (C_1); 75.8 (C_{D1}); 74.2 (C_2); 73.0 (C_3); 72.5 (C_5); 72.3 (C_4); 70.8 (C_7); 70.0 (D_2); 56.7 (C_{13}); 52.9 (C_6); 52.6 (C_{10}); 44.6 (C_8); 40.4, 40.2 (C_{D4}, C_{D5}); 29.3, 28.4 (C_{D3}, C_{D6}).

2.4.9.12 1,2-Cyclohexanedicarboxylic acid, 4-(2-azidoethoxy)-5-(6-(4-hydroxymethylenetriazol-1-yl)-α-D-6-deoxymannopyranosyloxy)-, 1,2-dimethyl ester, (1*S*,2*S*,4*S*,5*S*), 2.48d

To a solution of **2.55** (13.7 mg, 0.025 mmol, 1 eq) in DMF (0.3 ml) sodium azide (8.2 mg, 0.127 mmol, 5 eq.) was added. The reaction was stirred at 50°C for 4 days. The solvent was removed at reduced pressure and the crude residue was purified by flash chromatography (silica, chloroform with gradient of methanol from 3% to 30%) to afford 12.4 mg of product.



2.48d

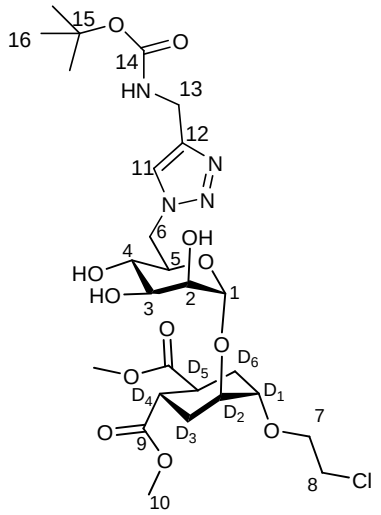
Yield = 90 %;

^{1}H NMR (400 MHz, CD_3OD): δ = 7.95 (s, 1H, H_{11}), 4.95 – 4.85 (m, 2H, H_1, H_{6a}), 4.68 (s, 2H, H_{13}), 4.47 (dd, 1H, H_{6b}, $J_{6a\text{-}6b}$ = 14.2 Hz, $J_{6b\text{-}5}$ = 9.3 Hz), 3.89 (dd, 1H, H_2, $J_{1\text{-}2}$ = 1.9 Hz, $J_{2\text{-}3}$ = 9.6 Hz), 3.87 – 3.84 (m, 1H, H_5), 3.73 (dd, 1H, H_3, $J_{3\text{-}4}$= 3.3 Hz, $J_{2\text{-}3}$ = 9.4 Hz), 3.67 – 3.50 (m, 8H, H_{10}, D_2, H_4), 3.54 – 3.47 (m, 1H, H_{7a}), 3.46 – 3.38 (m, 1H, H_{7b}), 3.37 – 3.30 (m, 2H, H_8), 3.19 – 3.13 (m, 1H, D_1), 2.91 – 2.73 (m, 2H, D_4, D_5), 2.03 – 1.93 (m, 2H, D_{3ax}, D_{6ax}), 1.77 – 1.55 (m, 2H, D_{3eq}, D_{6eq}).

^{13}C NMR (100 MHz, CD_3OD): δ = 177.1, 176.8 (C_9); 149.0 (C_{12}); 125.8 (C_{11}); 100.9 (C_1); 76.0 (C_{D1}); 74.2 (C_2); 72.9 (C_3); 72.5 (C_5); 72.3 (C_4); 70.0 (D_2); 69.7 (C_7); 56.7 (C_{13}); 53.0 (C_6); 52.6, 52.5 (C_{10}); 52.2 (C_8); 40.4, 40.1 (C_{D4}, C_{D5}); 29.1, 28.3 (C_{D3}, C_{D6}).

2.4.9.13 1,2-Cyclohexanedicarboxylic acid, 4-(2-chloroethoxy)-5-(6-(4-(N-carbotertbutoxy)methylene)triazol-1-yl)-α-D-6-deoxymannopyranosyloxy)-1,2-dimethyl ester, (1*S*,2*S*,4*S*,5*S*), 2.59

To a solution of N-Boc propargyl amine (7 mg, 0.124 mmol, 2 eq) in THF (0.5 ml) and water (0.5 ml) TBTA (6.4 mg, 0.025 mmol, 0.4 eq.), copper(II) sulphate (1.5 mg, 0.006 mmol, 0.1 eq.) and sodium ascorbate (5 mg, 0.025 mmol, 0.4 eq.) were added. The reaction was stirred at room temperature for 10 min then **2.55** was added (30 mg, 0.062 mmol, 1 eq.). The reaction was stirred for 4 h at room temperature under nitrogen. The solvent was removed at reduced pressure and the crude residue was purified by flash chromatography (silica, chloroform with gradient of methanol from 3% to 15%) to afford 36 mg of product.



2.59

Yield = 93 %

MS (ESI) calculated for: $[C_{26}H_{41}ClN_4O_{12}Na]^+$: 660.1; found: 559.3

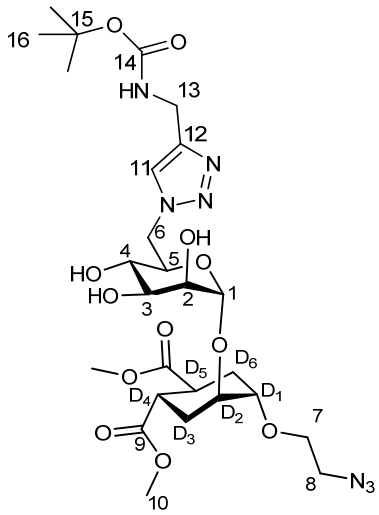
^{1}H NMR (400 MHz, CD_3OD): δ = 7.85 (s, 1H, H_{11}), 4.95 – 4.80 (m, 2H, H_1, H_{6a}), 4.43 (dd, 1H, H_{6b}, $J_{6a\text{-}6b}$ = 14.2 Hz, $J_{6b\text{-}5}$ = 9.5 Hz), 4.30 (s, 2H, H_{13}), 3.80 - 3.78 (m, 2H, H_5, H_2), 3.71 (dd, 1H, H_3, $J_{3.4}$ = 3.2 Hz, $J_{3\text{-}2}$ = 9.4 Hz), 3.67 – 3.42 (m, 12H, H_{10}, D_2, H_7, H_4, H_8), 3.18 – 3.11 (m, 1H,

D_1), 2.89 – 2.72 (m, 2H, D_4, D_5), 2.05 – 1.87 (m, 2H, D_{3ax}, D_{6ax}), 1.76 – 1.55 (m, 2H, D_{3eq}, D_{6eq}), 1.45 (s 9H, H_{16}),

^{13}C NMR (100 MHz, CD_3OD): δ = 177.1, 176.8 (C_9); 147.0 (C_{12}); 125.9 (C_{11}); 100.6 (C_1); 80.6 (C_{15}); 75.8 (C_{D1}); 74.1 (C_2); 72.5 (C_3); 72.4 (C_5); 72.3 (C_4); 70.8 (C_7); 70.0 (D_2); 52.9 (C_6); 52.6 (C_{10}); 44.7 (C_8); 40.4, 40.2 (C_{D4}, C_{D5}); 36.9 (C_{13}); 29.2 (C_{D3} or C_{D6}); 28.9 (C_{16}); 28.3 (C_{D3} or C_{D6}).

2.4.9.14 1,2-Cyclohexanedicarboxylic acid, 4-(2-azidoethoxy)-5-(6-(4-(N-carbotertbutoxy)methylene)triazol-1-yl)-α-D-6-deoxymannopyranosyloxy)-1,2-dimethyl ester, (1*S*,2*S*,4*S*,5*S*), 2.60

To a solution of **2.59** (35 mg, 0.0255 mmol, 1 eq) in DMF (0.5 ml) sodium azide (25 mg, 0.384 mmol, 7 eq.) was added. The reaction was stirred at 50°C for 4 days. The solvent was removed at reduced pressure and the crude residue was purified by flash chromatography (silica, chloroform with gradient of methanol from 2% to 15%) to afford 30 mg of product.



2.60

Yield = 85 %

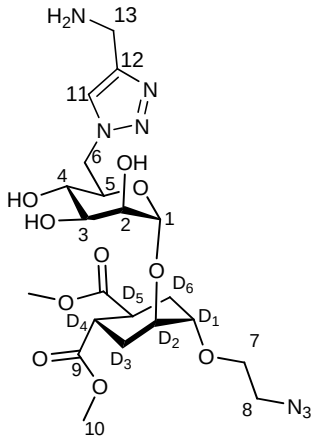
MS (ESI) calculated for: $C_{26}H_{41}N_7O_{12}Na]^+$: 666.6; found: 666.3

^{1}H NMR (400 MHz, CD_3OD): δ = 7.86 (s, 1H, H_{11}), 4.92 – 4.79 (m, 2H, H_1, H_{6a}), 4.43 (dd, 1H, H_{6b}, $J_{6a\text{-}6b}$ = 14.2 Hz, $J_{6b\text{-}5}$ = 9.5 Hz), 4.29 (s, 2H, H_{13}), 3.89 - 3.80 (m, 2H, H_5, H_2), 3.71 (dd, 1H, H_3, $J_{3.4}$ = 3.2 Hz, $J_{3\text{-}2}$ = 9.4 Hz), 3.65 (s, 3H, H_{10}), 3.63 (s, 3H, H_{10}), 3.60 - 3.52 (m, 2H, D_2, H_4), 3.51 – 3.43 (m, 1H, H_{7a}), 3.47 – 3.33 (m, 3H, H_{7b}, H_8), 3.19 – 3.13 (m, 1H, D_1), 2.94 – 2.72 (m, 2H, D_4, D_5), 2.03 – 1.93 (m, 2H, D_{3ax}, D_{6ax}), 1.77 – 1.55 (m, 2H, D_{3eq}, D_{6eq}), 1.45 (s 9H, H_{16}).

^{13}C NMR (100 MHz, CD_3OD): δ = 177.1, 176.7 (C_9); 147.0 (C_{12}); 125.9 (C_{11}); 100.6 (C_1); 80.6 (C_{15}); 75.8 (C_{D1}); 74.1 (C_2); 72.4 (C_3, C_5); 72.3 (C_4); 70.0 (D_2); 69.7 (C_7); 52.9 (C_6); 52.6, 52.5 (C_{10}); 52.2 (C_8); 40.4, 40.1 (C_{D4}, C_{D5}); 36.9 (C_{13}); 29.1 (C_{D3} or C_{D6}); 28.9 (C_{16}); 28.1 (C_{D3} or C_{D6}).

2.4.9.15 1,2-Cyclohexanedicarboxylic acid, 4-(2-azidoethoxy)-5-(6-((4-aminomethylene)triazol-1-yl)-α-D-6-deoxymannopyranosyloxy)-, 1,2-dimethyl ester, (1*S*,2*S*,4*S*,5*S*), 2.48e

2.60 (30 mg, 0.046 mmol, 1 eq.) was dissolved in TFA (1 ml). The resulting solution was stirred at 35°C for 20 minutes. The solvent was removed under reduced pressure and the crude residue was washed twice with a small amount diethyl ether. The product was dried under reduced pressure to afford 25 mg of product.



2.48e

Yield = 98 %;

$[\alpha]_D^{20}$ = + 14.4 (c = 0.22 in methanol);

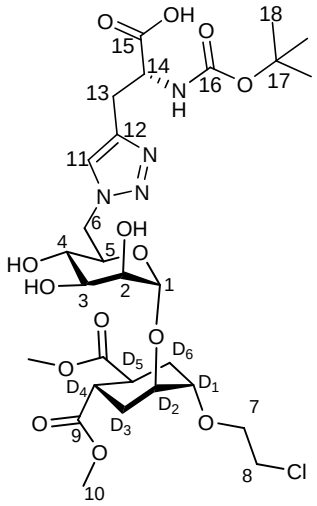
MS (HRMS) calculated for $[C_{21}H_{33}N_7O_{10}Na]^+$: 566.21866; found: 566.22022

^{1}H NMR (400 MHz, CD_3OD): δ = 8.08 (s, 1H, H_{11}), 4.90 – 4.79 (m, 2H, H_1, H_{6a}), 4.56 (dd, 1H, H_{6b}, $J_{6a\text{-}6b}$ = 14.2 Hz, $J_{6b\text{-}5}$ = 8.5 Hz), 4.25 (s, 2H, H_{13}), 3.97 - 3.87 (m, 1H, H_5), 3.85 (dd, 1H, H_2, $J_{1\text{-}2}$ = 1.7 Hz, $J_{2\text{-}3}$ = 3.2 Hz), 3.75 - 3.69 (m, 1H, H_3), 3.68 - 3.57 (m, 8H, H_{10}, D_2, H_{7a}), 3.56 – 3.45 (m, 2H, H_4, H_{7b}), 3.43 – 3.33 (m, 3H, H_8), 3.32 – 3.26 (m, 1H, D_1), 2.92 – 2.76 (m, 2H, D_4, D_5), 2.05 – 1.93 (m, 2H, D_{3ax}, D_{6ax}), 1.77 – 1.58 (m, 2H, D_{3eq}, D_{6eq}).

^{13}C NMR (100 MHz, CD_3OD): δ = 177.0, 176.7 (C_9); 141.0 (C_{12}); 127.4 (C_{11}); 100.4 (C_1); 76.1 (C_{D1}); 73.6 (C_5); 72.4 (D_2); 72.4 (C_2), 72.2 (C_3); 69.7 (C_4); 69.6 (C_7); 52.9 (C_6); 52.6, 52.6 (C_{10}); 52.2 (C_8); 40.4, 40.2 (C_{D4}, C_{D5}); 35.6 (C_{13}); 28.9 (C_{D3}, C_{D6}).

2.4.9.16 Compound 2.61

To a solution of N-Boc-(L-)propargyl glycine (13.3 mg, 0.062 mmol, 1.5 eq) in THF (0.5 ml) and water (0.5 ml) TBTA (4.4 mg, 0.0083 mmol, 0.2 eq.), (0.5 mg, 0.002 mmol, 0.05 eq) and sodium ascorbate (3.3 mg, 0.0166 mmol, 0.4 eq.) were added. The reaction was stirred at room temperature for 10 min then **2.55** was added (20 mg, 0.041 mmol, 1 eq.). The reaction was stirred for 2 h at room temperature under nitrogen. TLC ($CHCl_3$:MeOH = 9:1 and 8:2) indicated only starting materials therefore another portions of copper(II) sulphate (0.05 eq) and sodium ascorbate (0.4 eq) were added and the reaction was stirred for additional 2 h. The solvent was removed at reduced pressure and the crude residue was purified by flash chromatography (silica, chloroform with gradient of methanol from 3% to 30%) to afford ca 8.5 mg of product containing some insoluble impurities.



2.61

Yield = ca 30 %

MS (ESI) calculated for: $[C_{28}H_{43}ClN_4O_{14}]^+$: 695.1; found: 693.1

^{1}H NMR (400 MHz, D_2O): δ = 7.90 (br s, 1H, H_{11}), 4.96 – 4.79 (m, 2H, H_1, H_{6a}), 4.45 (dd, 1H, H_{6b}, $J_{6a\text{-}6b}$ = 13.9 Hz, $J_{6b\text{-}5}$ = 9.5 Hz), 4.12 – 4.23 (m, 1H, H_{14}), 3.98 (dd, 1H, H_2, $J_{1\text{-}2}$ = 1.7 Hz, $J_{2\text{-}3}$ = 7.0 Hz), 3.91 - 3.81 (m, 2H, H_5, H_3), 3.73 – 3.59 (m, 9H, H_{10}, H_4, H_8), 3.60 – 3.53 (m, 2H, H_{7a}, D_2), 3.52 – 3.41 (m, 1H, H_{7b}), 3.27 – 3.17 (m, 1H, H_{13a}), 3.13 – 3.08 (m, 1H, D_1), 3.05 –

2.92 (m, 1H, H_{13b}), 2.93 – 2.79 (m, 2H, D_4, D_5), 2.13 – 1.83 (m, 2H, D_{3ax}, D_{6ax}), 1.79 – 1.55 (m, 2H, D_{3eq}, D_{6eq}), 1.36 (s, 9H, H_{18}),

^{13}C NMR (100 MHz, D_2O): δ = 179.2, 179.1 (C_9); 159.0 (C_{16}); 100.9 (C_1); 76.0 (C_{D1}); 74.2 (C_5); 72.5 (C_3); 73.5 (D_2); 72.2 (C_2); 72.0 (C_3); 70.8 (C_7); 70.0 (C_4); 54.5, 54.5 (C_{10}); 53.2 (C_6); 45.4 (C_8); 40.8 (C_{D4}, C_{D5}); 30.4 (C_{13}); 29.5 (C_{18}); 29.0, 28.6 (C_{D3}, C_{D6}).

2.5 References

1. Taylor, M. E.; Drickamer, K., Structural insights into what glycan arrays tell us about how glycan-binding proteins interact with their ligands. *Glycobiology* **2009,** *19* (11), 1155-1162.

2. Geijtenbeek, T. B. H.; van Vliet, S. J.; Koppel, E. A.; Sanchez-Hernandez, M.; Vandenbroucke-Grauls, C. M. J. E.; Appelmelk, B.; van Kooyk, Y., Mycobacteria target DC-SIGN to suppress dendritic cell function. *J Exp Med* **2003,** *197* (1), 7-17.

3. Borrok, M. J.; Kiessling, L. L., Non-carbohydrate inhibitors of the lectin DC-SIGN. *J Am Chem Soc* **2007,** *129* (42), 12780-12785.

4. Mangold, S. L.; Prost, L. R.; Kiessling, L. L., Quinoxalinone inhibitors of the lectin DC-SIGN. *Chemical Science* **2012,** *3* (3).

5. Timpano, G.; Tabarani, G.; Anderluh, M.; Invernizzi, D.; Vasile, F.; Potenza, D.; Nieto, P. M.; Rojo, J.; Fieschi, F.; Bernardi, A., Synthesis of novel DC-SIGN ligands with an alpha-fucosylamide anchor. *Chembiochem* **2008,** *9* (12), 1921-1930.

6. Andreini, M.; Doknic, D.; Sutkeviciute, I.; Reina, J. J.; Duan, J. X.; Chabrol, E.; Thepaut, M.; Moroni, E.; Doro, F.; Belvisi, L.; Weiser, J.; Rojo, J.; Fieschi, F.; Bernardi, A., Second generation of fucose-based DC-SIGN ligands: affinity improvement and specificity versus Langerin. *Org Biomol Chem* **2011,** *9* (16), 5778-5786.

7. Mitchell, D. A.; Jones, N. A.; Hunter, S. J.; Cook, J. M. D.; Jenkinson, S. F.; Wormald, M. R.; Dwek, R. A.; Fleet, G. W. J., Synthesis of 2-C-branched derivatives of D-mannose: 2-C-aminomethyl-D-mannose binds to the human C-type lectin DC-SIGN with affinity greater than an order of magnitude compared to that of D-mannose. *Tetrahedron-Asymmetr* **2007,** *18* (12), 1502-1510.

8. Maria, S.; Sanchez-Medina, I.; Mereghetti, P.; Belvisi, L.; Jimenez-Barbero, J.; Bernardi, A., Synthesis and conformational analysis of an alpha-D-mannopyranosyl(1 -> 2)-alpha-D-mannopyranosyl-(1 -> 6)-alpha-D-mannopyranose mimic. *Carbohyd Res* **2007,** *342* (12-13), 1859-1868.

9. Garber, K. C. A.; Wangkanont, K.; Carlson, E. E.; Kiessling, L. L., A general glycomimetic strategy yields non-carbohydrate inhibitors of DC-SIGN. *Chem Commun* **2010,** *46* (36), 6747-6749.

10. Reina, J. J.; Sattin, S.; Invernizzi, D.; Mari, S.; Martinez-Prats, L.; Tabarani, G.; Fieschi, F.; Delgado, R.; Nieto, P. M.; Rojo, J.; Bernardi, A., 1,2-mannobioside mimic: Synthesis, DC-SIGN interaction by NMR and docking, and antiviral activity. *Chemmedchem* **2007,** *2* (7), 1030-1036.

11. Obermajer, N.; Sattin, S.; Colombo, C.; Bruno, M.; Svajger, U.; Anderluh, M.; Bernardi, A., Design, synthesis and activity evaluation of mannose-based DC-SIGN antagonists. *Mol Divers* **2011,** *15* (2), 347-360.

12. Carbohydrate Multivalent System as Tools to Study Pathogen Interactions with DC-SIGN. http://www.carmusys.iiq.csic.es/.

13. Sattin, S. Synthesis of inhibitors of DC-SIGN mediated infections - PhD thesis. University Degli Studi di Milano, Milano, 2009.

14. Bernardi, A.; Arosio, D.; Manzoni, L.; Micheli, F.; Pasquarello, A.; Seneci, P., Stereoselective synthesis of conformationally constrained cyclohexanediols: A set of molecular scaffolds for the synthesis of glycomimetics. *J Org Chem* **2001,** *66* (19), 6209-6216.

15. Keicher, T.; Löbbecke, S., Lab-Scale Synthesis of Azido Compounds: Safety Measures and Analysis. In *Organic Azides*, John Wiley & Sons, Ltd: 2010; pp 1-27.

16. Lee, D. J.; Kowalczyk, R.; Muir, V. J.; Rendle, P. M.; Brimble, M. A., A comparative study of different glycosylation methods for the synthesis of D-mannopyranosides of N alpha-fluorenylmethoxycarbonyl-trans-4-hydroxy-L-proline allyl ester. *Carbohyd Res* **2007,** *342* (17), 2628-2634.

17. Schmidt, R. R.; Jung, K. H., *Preparative Carbohydrate Chemistry*. Marcel Dekker Inc.: New York, 1997.

18. Arce, E.; Nieto, P. M.; Diaz, V.; Castro, R. G.; Bernad, A.; Rojo, J., Glycodendritic structures based on Boltorn hyperbranched polymers and their interactions with Lens culinaris lectin. *Bioconjugate Chem* **2003,** *14* (4), 817-823.

19. Lasala, F.; Arce, E.; Otero, J. R.; Rojo, J.; Delgado, R., Mannosyl glycodendritic structure inhibits DC-SIGN-mediated Ebola virus infection in cis and in trans. *Antimicrob Agents Ch* **2003,** *47* (12), 3970-3972.

20. Dong, Y. H.; Liang, X. M.; Yuan, H. Z.; Qi, S. H.; Chen, F. H.; Wang, D. Q., Potential green fungicide: 16-oxo-1-oxa-4-azoniacyclohexadecan-4-ium tetrafluoroborate. *Green Chem* **2008,** *10* (9), 990-994.

21. Weissman, S. A.; Zewge, D.; Chen, C., Ligand-free palladium-catalyzed cyanation of aryl halides. *J Org Chem* **2005,** *70* (4), 1508-1510.

22. Andre, S.; Velasco-Torrijos, T.; Leyden, R.; Gouin, S.; Tosin, M.; Murphy, P. V.; Gabius, H. J., Phenylenediamine-based bivalent glycocyclophanes: synthesis and analysis of the influence of scaffold rigidity and ligand spacing on lectin binding in cell systems with different glycomic profiles. *Org Biomol Chem* **2009,** *7* (22), 4715-4725.

23. Leyden, R.; Velasco-Torrijos, T.; Andre, S.; Gouin, S.; Gabius, H. J.; Murphy, P. V., Synthesis of Bivalent Lactosides Based on Terephthalamide, N,N '-Diglucosylterephthalamide, and Glycophane Scaffolds and Assessment of Their Inhibitory Capacity on Medically Relevant Lectins. *J Org Chem* **2009,** *74* (23), 9010-9026.

24. Choi, H. J.; Park, Y. S.; Kim, M. G.; Park, Y. J.; Yoon, N. S.; Bell, T. W., A D-2 symmetric tetraamide macrocycle based on 1,1 ',4,4 '-tetrahydro[3,3 '(2H,2 ' H)-spirobiquinoline]-2,2 '-dione: synthesis and selectivity for lithium over sodium and alkaline earth ions. *Tetrahedron* **2006,** *62* (37), 8696-8701.

25. Cooper, M. A., Optical biosensors in drug discovery. *Nat Rev Drug Discov* **2002,** *1* (7), 515-528.

26. Halary, F.; Amara, A.; Lortat-Jacob, H.; Messerle, M.; Delaunay, T.; Houles, C.; Fieschi, F.; Arenzana-Seisdedos, F.; Moreau, J. F.; Dechanet-Merville, J., Human cytomegalovirus

binding to DC-SIGN is required for dendritic cell infection and target cell trans-infection. *Immunity* **2002,** *17* (5), 653-664.

27. Fieschi, F., IBS/LPM; 41 rue Jules Horowitz, 38027 Grenoble Cedex 1 - France;Tel : 33 (0)4 38 78 91 77, Fax : 33 (0)4 38 78 54 94.

28. Tabarani, G.; Thepaut, M.; Stroebel, D.; Ebel, C.; Vives, C.; Vachette, P.; Durand, D.; Fieschi, F., DC-SIGN Neck Domain Is a pH-sensor Controlling Oligomerization SAXS and hydrodynamic studies of extracellular domain. *J Biol Chem* **2009,** *284* (32), 21229-21240.

29. Thépaut, M.; Guzzi, C.; Sutkeviciute, I.; Sattin, S.; Ribeiro-Viana, R.; Varga, N.; Chabrol, E.; Rojo, J.; Angulo, J.; Bernardi, A.; Nieto, P. M.; Fieschi, F., Structure of a glycomimetic ligand in the Carbohydrate Recognition Domain of C-type lectin DC-SIGN. Structural requirements for selectivity and ligand design. *Submited* **2012**.

30. Ciobanu, M.; Huang, K. T.; Daguer, J. P.; Barluenga, S.; Chaloin, O.; Schaeffer, E.; Mueller, C. G.; Mitchell, D. A.; Winssinger, N., Selection of a synthetic glycan oligomer from a library of DNA-templated fragments against DC-SIGN and inhibition of HIV gp120 binding to dendritic cells. *Chem Commun* **2011,** *47* (33), 9321-9323.

31. Gorska, K.; Manicardi, A.; Barluenga, S.; Winssinger, N., DNA-templated release of functional molecules with an azide-reduction-triggered immolative linker. *Chem Commun* **2011,** *47* (15), 4364-4366.

32. Feinberg, H.; Taylor, M. E.; Razi, N.; McBride, R.; Knirel, Y. A.; Graham, S. A.; Drickamer, K.; Weis, W. I., Structural Basis for Langerin Recognition of Diverse Pathogen and Mammalian Glycans through a Single Binding Site. *J Mol Biol* **2011,** *405* (4), 1027-1039.

33. Neumann, J.; Weingarten, S.; Thiem, J., Synthesis of novel di- and trisaccharide mimetics with non-glycosidic amino bridges. *Eur J Org Chem* **2007,** (7), 1130-1144.

34. Green, D. E.; Ferreira, C. L.; Stick, R. V.; Patrick, B. O.; Adam, M. J.; Orvig, C., Carbohydrate-bearing 3-hydroxy-4-pyridinonato complexes of gallium(III) and indium(III). *Bioconjugate Chem* **2005,** *16* (6), 1597-1609.

35. Meldal, M.; Tornoe, C. W., Cu-catalyzed azide-alkyne cycloaddition. *Chem Rev* **2008,** *108* (8), 2952-3015.

36. Sharpless, K. B.; Kolb, H. C., Click chemistry. A concept for merging process and discovery chemistry. *Abstr Pap Am Chem S* **1999,** *217*, U95-U95.

37. Kolb, H. C.; Finn, M. G.; Sharpless, K. B., Click chemistry: Diverse chemical function from a few good reactions. *Angew Chem Int Edit* **2001,** *40* (11), 2004-+.

38. Chan, T. R.; Hilgraf, R.; Sharpless, K. B.; Fokin, V. V., Polytriazoles as Copper(I)-Stabilizing Ligands in Catalysis. *Org Lett* **2004,** *6* (17), 2853-2855.

39. Tsegaye, T. S.; Pohlmann, S., The multiple facets of HIV attachment to dendritic cell lectins. *Cell Microbiol* **2010,** *12* (11), 1553-1561.

40. Valladeau, J.; Ravel, O.; Dezutter-Dambuyant, C.; Moore, K.; Kleijmeer, M.; Liu, Y.; Duvert-Frances, V.; Vincent, C.; Schmitt, D.; Davoust, J.; Caux, C.; Lebecque, S.; Saeland, S.,

Langerin, a novel C-type lectin specific to Langerhans cells, is an endocytic receptor that induces the formation of Birbeck granules. *Immunity* **2000,** *12* (1), 71-81.

41. Menon, S.; Rosenberg, K.; Graham, S. A.; Ward, E. M.; Taylor, M. E.; Drickamer, K.; Leckband, D. E., Binding-site geometry and flexibility in DC-SIGN demonstrated with surface force measurements. *P Natl Acad Sci USA* **2009,** *106* (28), 11524-11529.

42. Chatwell, L.; Holla, A.; Kaufer, B. B.; Skerra, A., The caxbohydrate recognition domain of Langerin reveals high structural similarity with the one of DC-SIGN but an additional, calcium-independent sugar-binding site. *Mol Immunol* **2008,** *45* (7), 1981-1994.

43. Tanaka, M.; Anan, K.; Demizu, Y.; Kurihara, M.; Doi, M.; Suemune, H., Side-Chain Chiral Centers of Amino Acid and Helical-Screw Handedness of Its Peptides. *J Am Chem Soc* **2005,** *127* (33), 11570-11571.

44. Keng, P. Y.; Shim, I.; Korth, B. D.; Douglas, J. F.; Pyun, J., Synthes is and self-assembly of polymer-coated ferromagnetic nanoparticles. *Acs Nano* **2007,** *1* (4), 279-292.

45. Salome, C.; Salome-Grosjean, E.; Park, K. D.; Morieux, P.; Swendiman, R.; DeMarco, E.; Stables, J. P.; Kohn, H., Synthesis and Anticonvulsant Activities of (R)-N-(4 '-Substituted)benzyl 2-Acetamido-3-methoxypropionamides. *J Med Chem* **2010,** *53* (3), 1288-1305.

46. Dogan, Z.; Paulini, R.; Stutz, J. A. R.; Narayanan, S.; Richert, C., 5 '-Tethered stilbene derivatives as fidelity- and affinity-enhancing modulators of DNA duplex stability. *J Am Chem Soc* **2004,** *126* (15), 4762-4763.

47. Tucker, J. L.; Couturier, M.; Castaldi, M. J.; Chiu, C. K. F.; Gestmann, D., Scalable process for 4-(2-hydroxy-2-methyl)-ethyl-benzylamine. *Synthetic Commun* **2006,** *36* (15), 2145-2150.

48. Hyder, Z.; Ruan, J. W.; Xiao, J. L., Hydrogen-bond-directed catalysis: Faster, regioselective and cleaner Heck arylation of electron-rich olefins in alcohols. *Chem-Eur J* **2008,** *14* (18), 5555-5566.

49. Langhals, H.; Obermeier, A.; Floredo, Y.; Zanelli, A.; Flamigni, L., Light-Driven Charge Separation in Isoxazolidine-Perylene Bisimide Dyads. *Chem-Eur J* **2009,** *15* (46), 12733-12744.

50. Martinelli, J. R.; Watson, D. A.; Freckmann, D. M. M.; Barder, T. E.; Buchwald, S. L., Palladium-catalyzed carbonylation reactions of aryl bromides at atmospheric pressure: A general system based on xantphos. *J Org Chem* **2008,** *73* (18), 7102-7107.

Chapter 3

Multivalent glycoconjugate systems

3.1 Principles of multivalent structures in biological systems and drug design

The ability of glycans to encode biochemical information has recently emerged and its unraveling has been acknowledged as one of the most critical challenges for the postgenomic era.[1,2,3] The complexity of the problem has often been summarized: carbohydrates are the most abundant type of biomolecule in nature. They are widely expressed as glycolipids and glycoproteins, and glycosylation is the most widespread post-translational modification of proteins. Furthermore, glycans are more complex and difficult to analyze and synthesize than other macromolecules. Therefore, despite numerous efforts, the extent to which the sugar code has been deciphered is still limited. Nonetheless, it is clearly emerging that many fundamental biological processes are controlled by sugar-mediated information, among them: quality control of protein folding, intra- and extracellular trafficking of glycoconjugates, signalling, host defence pathways, modulation of cell–cell and cell–matrix adhesion, both in physiological situations (as egg-sperm interaction, embryogenesis, etc.) and in pathological conditions (inflammation, cancer, etc).[4] Most of the sugar controlled processes identified so far involve polyvalent interactions of glycoconjugates with polyvalent (lectins) proteins. Polyvalent interactions are characterized by the simultaneous binding of multiple ligands on one biological entity (a molecule, a surface) to multiple receptors on another.[5] These interactions occur throughout Nature, and have unique features that monovalent interactions do not share. In particular, polyvalent interactions can be collectively much stronger than the corresponding monovalent interactions: this is exactly the case for carbohydrates. Carbohydrates tend to bind only weakly to their complementary proteins[6] and stronger binding or enhanced inhibition is often achieved by multiple interactions by multivalent carbohydrates compounds.

There are mainly two different mechanisms of polyvalent binding that can be identified:

Chelation

If the protein receptor allows simultaneous binding of more than one ligand of a multivalent system to more than one binding site of the protein target, the binding of the second ligand should be enhanced, since translational and rotational entropic penalties were already paid for by the first binding event (Figure **3.1 A**). Chelation can also occur by two non-identical ligands to two non-identical binding sites (Figure **3.1 B**). Specially in this case, the nature of the spacer separating the ligands is of great importance: enhancements can be as high as 10^3–10^6 fold, for both carbohydrate based systems[7] and non-carbohydrate systems.[8]

Statistical rebinding[9]

When the tether between the ligands is too short to allow chelation or when the protein contains only a single binding site and multivalency enhancements are observed nonetheless, one of the active mechanisms can be named statistical rebinding. This effect, also called proximity/statistical effect, is caused by the slower off-rate of the multivalent carbohydrate in comparison with a monovalent ligand, due to the close proximity of additional ligands that can take the place of the first one after it is released, resulting in a net increased affinity (Figure **3.1 C**). In general, non-chelation effects are typically smaller than those observed where a chelation mechanism is operating. However, when the multivalent ligands are large and contain many ligand copies, the effects can be large as well.

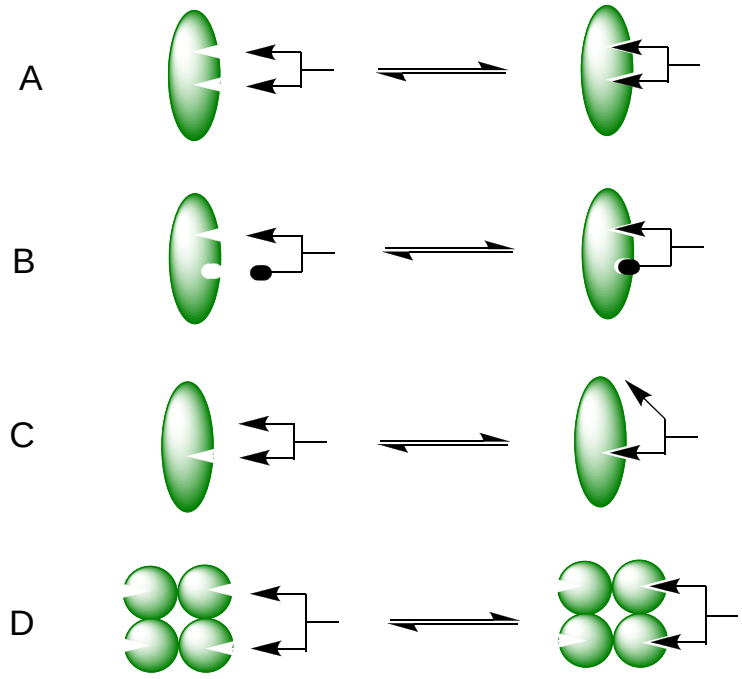
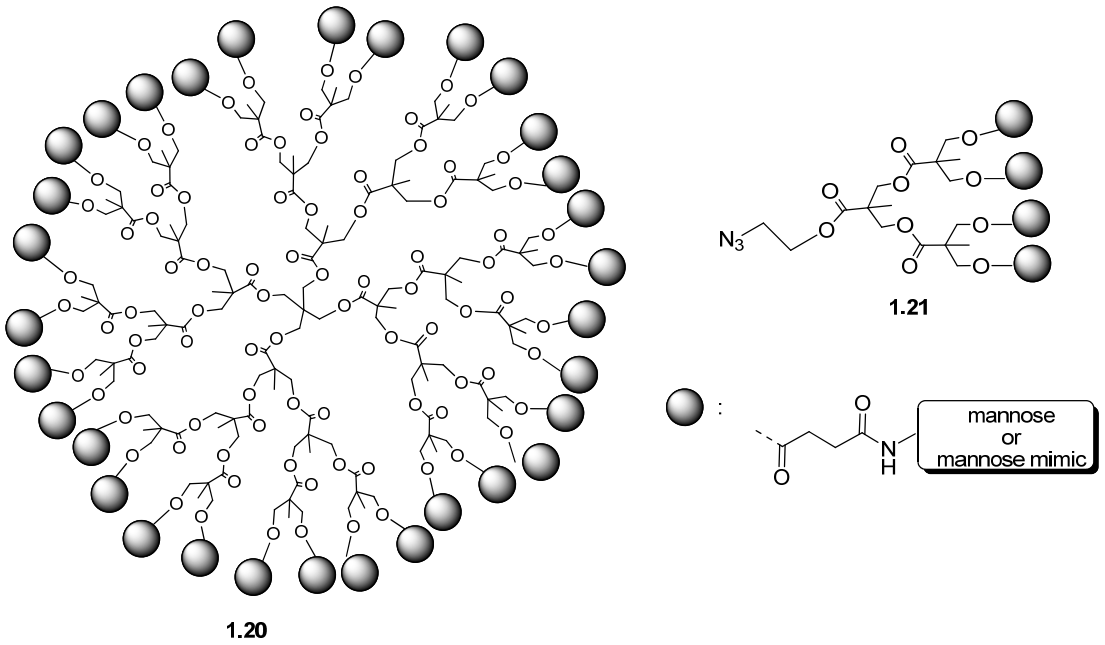


Figure 3.1 Schematic representation of the main polyvalent binding mechanisms

Both these mechanisms can contribute to binding in the case of multimeric lectins (Figure **3.1D**) depending on the ligand considered. This is the situation operating with DC-SIGN, which **i**s generally presented as a tetramer (see introduction) and with other known lectins**.**

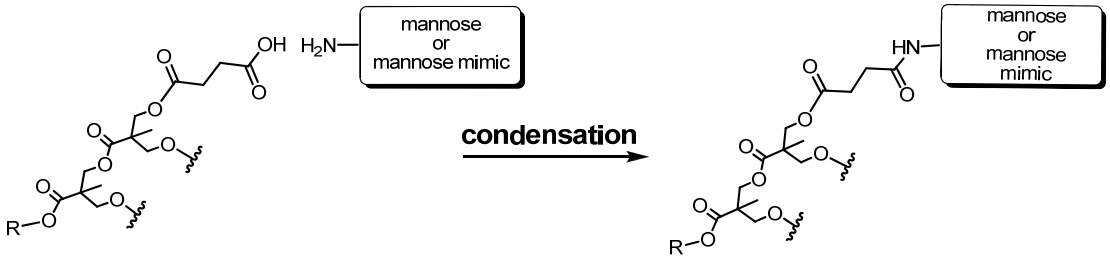
In biological settings, the situation can be further complicated by the density of the multimeric receptor on the cell membrane. In case of a high density, a ligand which may be too small to reach two adjacent binding sites in the same protein, may be able to bridge across two different receptor molecules, adding a further layer of complexity to the system.

In the first chapter a brief review of some of the multivalent compounds which target DC-SIGN are described.[10,11,12,13,14,15] From the reported results is clear that the principle of multivalency is used with great success in the development of antagonist of lectins such as DC-SIGN. Multi and polyvalent structures bearing sugars or sugar mimics were previously prepared also in our group (prof. Anna Bernardi, Scheme 3.1). [11,12]



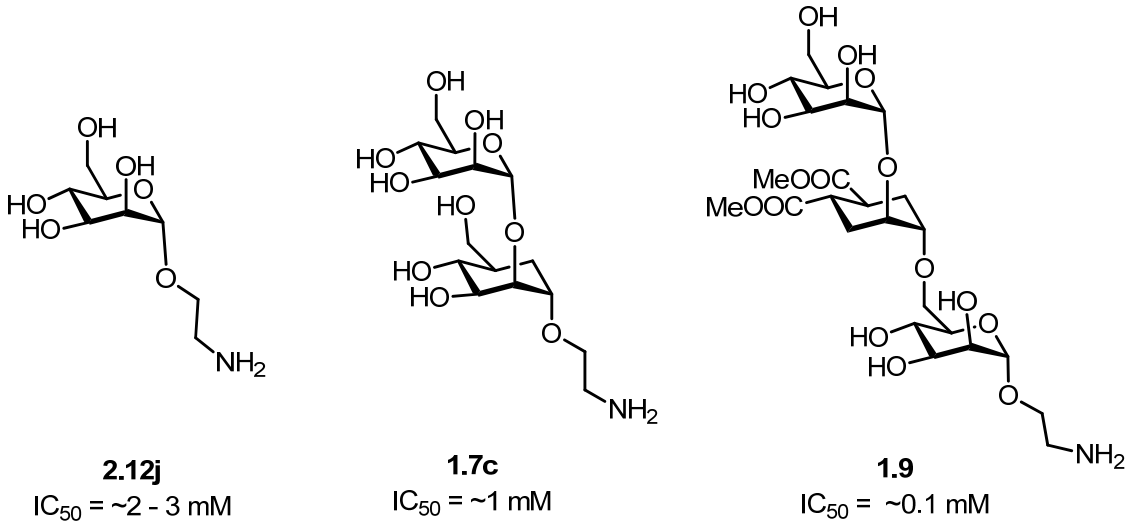
Scheme 3.1 Boltorn type dendrimer **1.20** and dendron **1.21** decorated with mannose or mannose-based DC-SIGN inhibitors[10,11,12]

The two main polyvalent scaffolds used in the DC-SIGN project were a Boltorn type dendrimer **1.20** able to conjugate 32 copies of a monovalent ligand, and a tetravalent dendron **1.21** also derived from 2,2-bishydroxymethylene propanoic acid, which could be loaded with four monovalent ligands. Both of them have a polyester backbone which possesses relatively good flexibility and water solubility, while the outer shelves are functionalized with carboxylic groups. The conjugation occurs via amide bond formation between the amines of the monovalent ligands and carboxylic acids of the corresponding scaffolds (Scheme 3.2).



Scheme 3.2 Functionalisation of polyvalent scaffolds **1.20** and **1.21** with a corresponding monovalent ligand

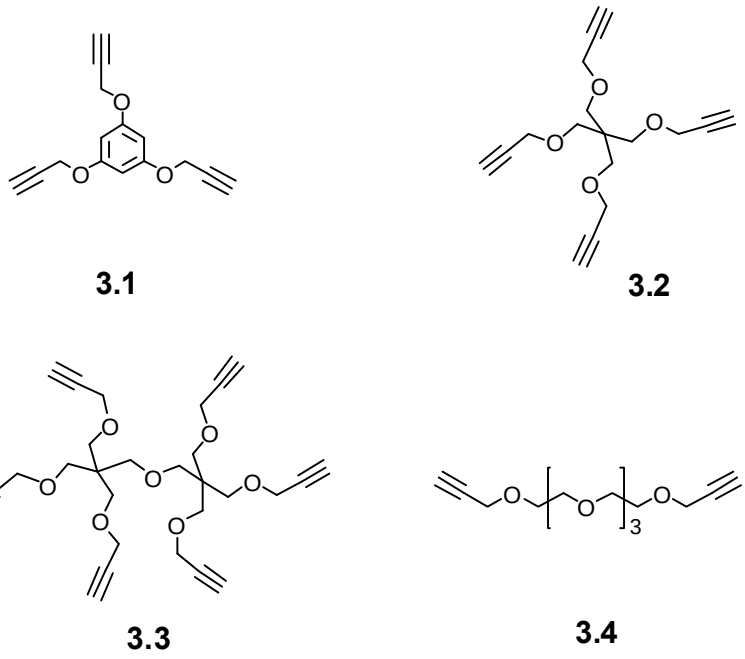
Compounds such as (2-aminoethyl)-α-D-mannose **2.12j**, or the mimics **1.7c**[16] and **1.9**[17] were used as monovalent ligands during the preparation of polyvalent structures (Scheme 3.3).



Scheme 3.3 Mannose based monovalent DC-SIGN ligands **2.2j**, **1.7c** and **1.9**

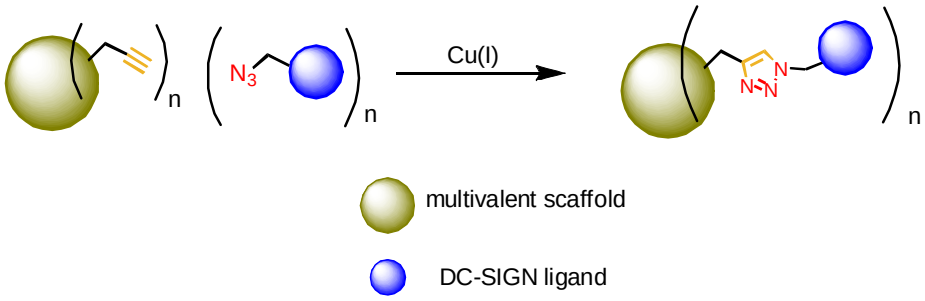
3.2 Goal of the study

Structures **1.20** and **1.21** demonstrated that going from mono to multivalent presentation is a promising way to improve the affinity of our ligands. However, during the preparation of these structures some drawbacks, such as relatively long synthesis and chemical instability of the scaffolds, were observed. Therefore, to overcome this problem, a set of new multivalent structures were proposed (Scheme 3.4). The synthesis and development of these molecules was carried out in collaboration with the group of Dr. Javier Rojo (Seville, Spain)[18]. Several, structurally similar multivalent scaffolds have been proposed differing in the number of possible functionalization (valency).



Scheme 3.4 Multivalent structure developed in the laboratory of Dr. Javier Rojo

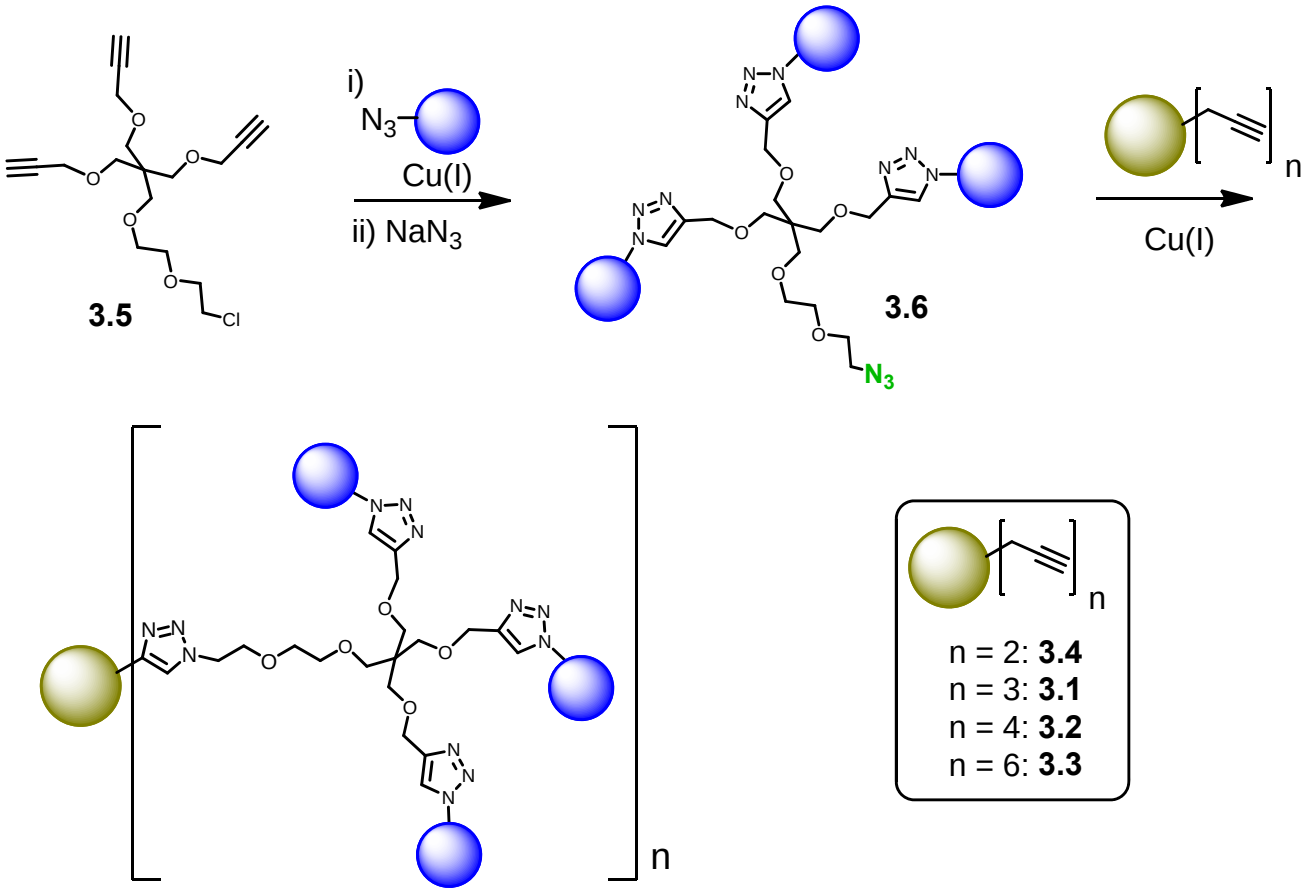
The designed molecules have chemically solid backbone decorated with terminal triple bonds which allow functionalization with monovalent ligands having an azide function (Scheme 3.5). The corresponding reaction is a copper(I) catalyzed dipolar cycloaddition also known as click reaction (described more in details in the following section).



Scheme 3.5 Functionalisation of polyvalent scaffolds via 1,3 dipolar cycloadition

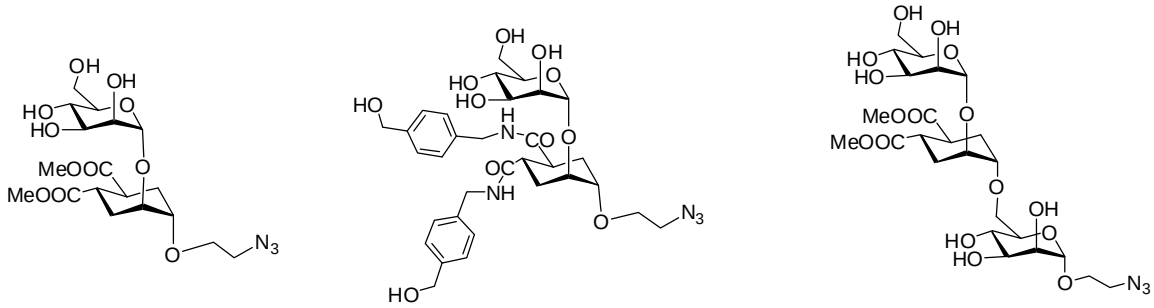
The three basic structures **3.1**,[19] **3.2**[20] and **3.3** can lead to a tri, tetra and hexavalent presentation of a corresponding monovalent ligand. Further, a trivalent scaffold **3.5**[21] was proposed, which can be functionalized with three copies of a ligand and, after transformation of the chloride on the tail to an azide, to give molecule **3.6**. Dendron **3.6** can be connected to scaffolds such as **3.1, 3.2, 3.3** and **3.4** leading to compounds with higher valency and different shape (Scheme 3.6). For

instance, after the functionalization of molecule **3.4**[22] with dendron **3.6** an elongated hexavalent system can be obtained.



Scheme 3.6 Synthesis of dendron **3.6** and its use in the preparation of multivalent ligands with higher valency.

Mannose mimetics **1.7b**, **2.2f** and **1.9** (described in chapter 1 and 2) we selected as monovalent ligands for conjugation with the multivalent scaffolds mentioned above. The IC50 activities of these molecules measured by SPR competition are ranging from 0.1 Mm to 0.6 mM, which allows us to study the impact of the potency of a monovalent lingand on the multivalency effect.



1.7b
IC50 = ~0.6 mM

2.2f
IC50 = ~0.2 mM

1.9
IC50 = ~0.1 mM

Scheme 3.7 Monovalent mannose based DC-SIGN ligand

The general goal is to synthesize a small library of multivalent compounds using different scaffolds (Scheme 3.4 and 3.6) and ligands (Scheme 3.7) in order to investigate which multivalent structures have the best properties in terms of activity, stability and solubility.
In order to learn the basic techniques required during the synthesis of multivalent compounds, I was seconded for 3 months in the group of Dr. Javier Rojo[18] in Seville. Some of the multivalent compounds described below were prepared during this period, whereas others were prepared in the group of Anna Bernardi (Milano) after my return.

In addition to the scaffolds discussed so far, a new approach of binding DC-SIGN in the multivalent fashion is discussed. Based on the fact that DC-SIGN is a homo tetramer with known distances between the binding sites (35 – 38 Å)[23], a multivalent structure with proper size and shape could bind several binding sites simultaneously. The concept of inhibition of multiple binding sites by one molecule has been previously found as an efficient way to inhibit certain proteins.[7,24] To achieve the same goal with DC-SIGN, multivalent structures must be designed for this purpose. We thought that elongated molecules functionalized with DC-SIGN ligands at the two terminals could reach two binding sites within one CRD (Figure 3.2). The spacer should be rigid to control the position of the ligands relative to one another, as well as the distance between them. Furthermore, in order to allow some flexibility in the system and to exploit also a statistical rebinding mechanism, the two ends of the spacer should be decorated with small, flexible dendrons.

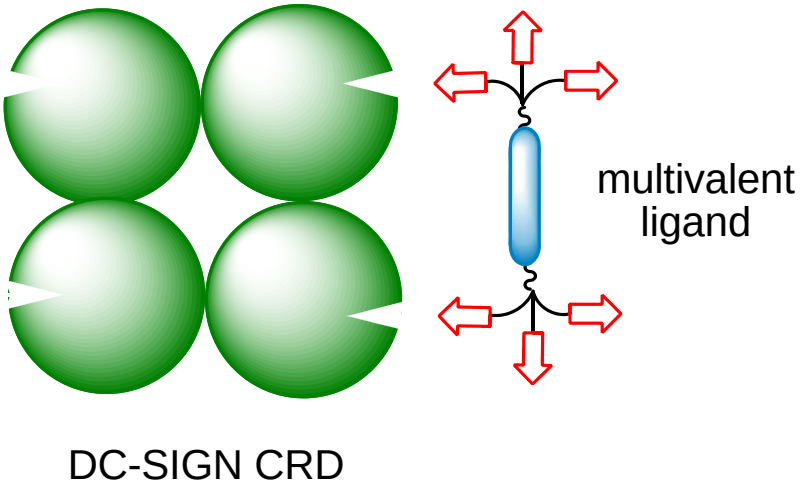
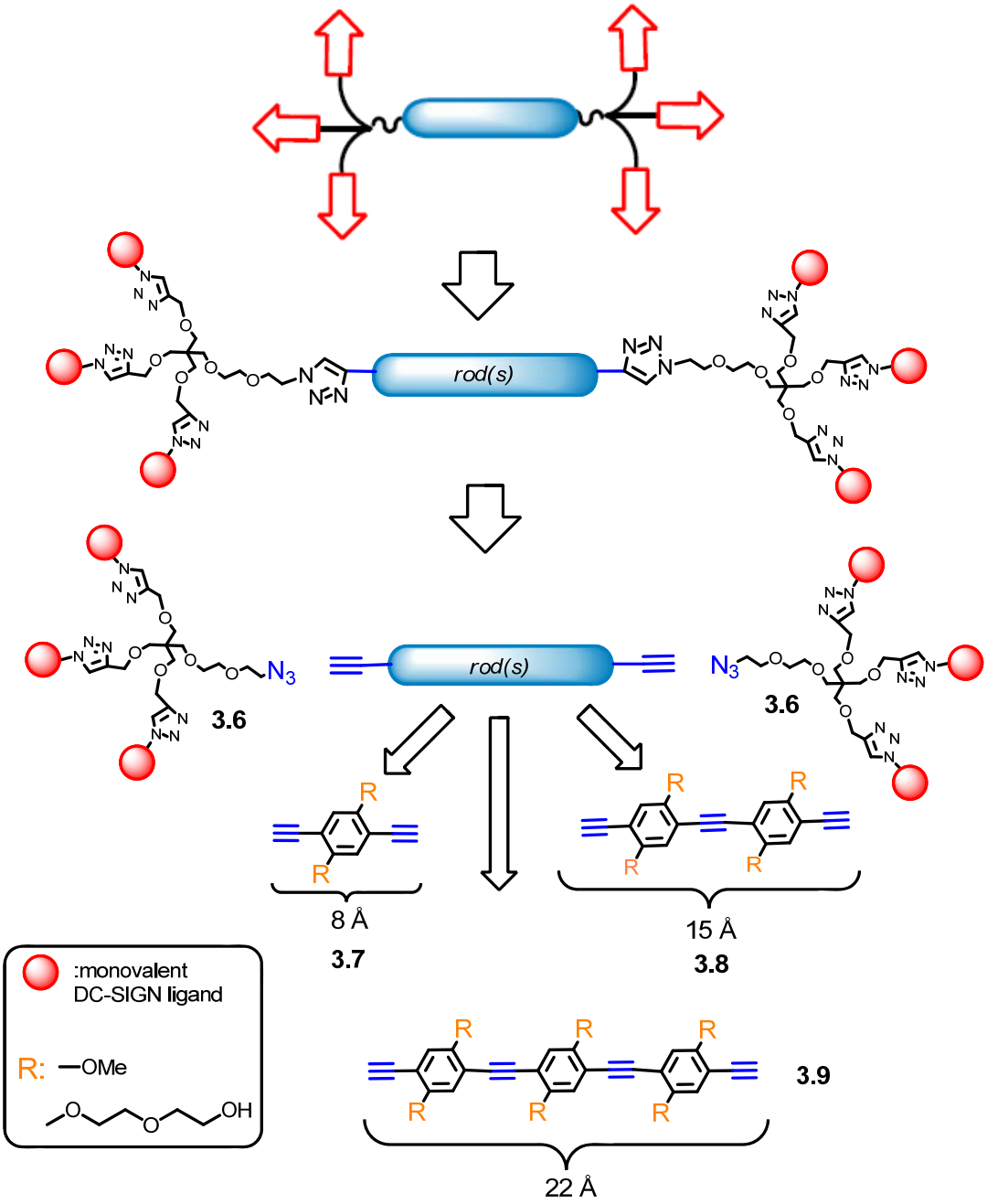


Figure 3.2 The concept of the inhibition of two binding sites within one DC-SIGN CRD one multivalent molecule

The rigidity of the spacer could be achieved using aromatic rings connected via triple bonds (Scheme 3.8). By preparing "molecular rods",[25] with different number of aromatic-alkyne unit, a set of rigid spacers with different lengths can be generated. However, to avoid solubility problems (first of all in water) the spacers have to be decorated with functional groups which would help to solubilize the final molecule. As already mentioned, the two terminals of the molecular rods are functionalized with triple bonds which allow conjugation with molecules such as **3.6**, bearing previously tested monovalent ligands (Scheme 3.8). The final structures consist of a rigid spacer functionalized with two flexible dendrons. A molecule of this type has the potential of combining simultaneous binding of two DC-SIGN binding sites (chelation) with proximity effects generated at each binding site by the trivalent dendron (statistical rebinding).



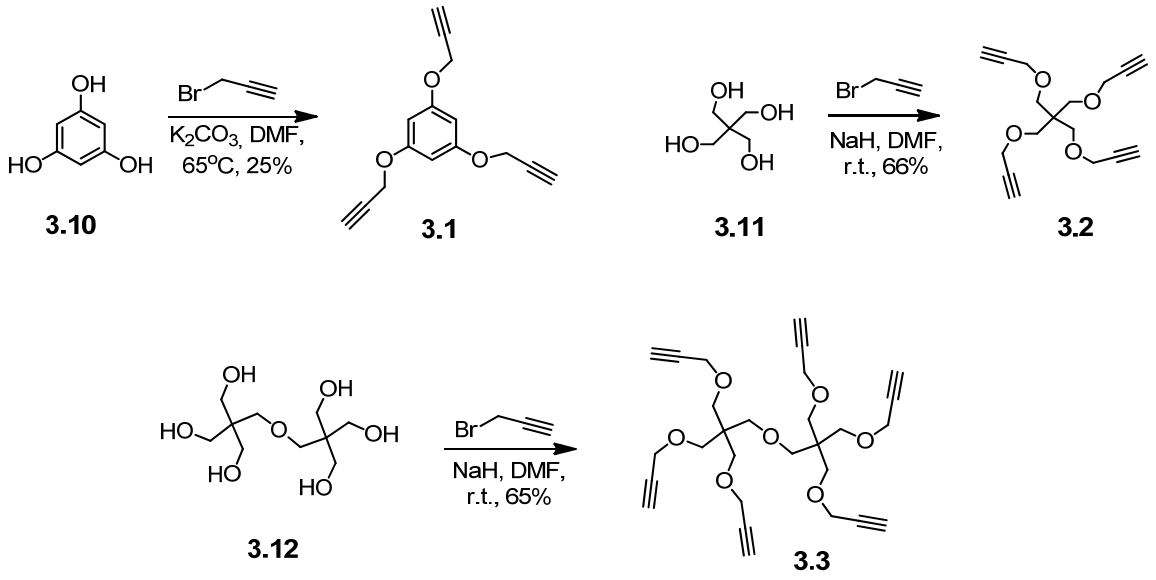
Scheme 3.8 The strategy of preparation of molecular rods targeting DC-SIGN

Since the final molecules are relatively rigid, the distances between the monovalent ligands of each dendrimer play a crucial role. By preparing a set of aromatic rods **3.7**, **3.8** and **3.9** the overall length of the final molecule can be efficiently tuned. In order to know which molecule among the proposed ones is the most promising candidate to inhibit two binding sites simultaneously, molecular modeling and docking studies have been performed in collaboration with computational chemists within the group.[26]

3.3 Synthesis of multivalent glycoconjugates

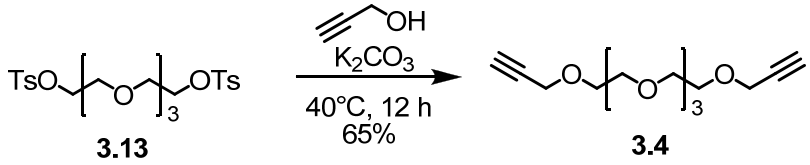
3.3.1 Synthesis of multivalent scaffolds

The synthesis of structures **3.1** - **3.5** mentioned in the previous chapter was developed in the laboratory of Dr. Javier Rojo by a PhD student, Renato Ribeiro (collaboration within CARMUSYS)[27]. The synthesis of multivalent scaffolds used in this study is simple and much more efficient in comparison with the preparation of dendrimers **1.20** and **1.21**. Compounds **3.1**,[20] **3.2**[19] and **3.3** can be prepared in one step starting from commercially available starting materials. The general strategy is based on the treatment of polyalcohols **3.10** - **3.12** with a base in the presence of propargyl bromide (Scheme 3.9).



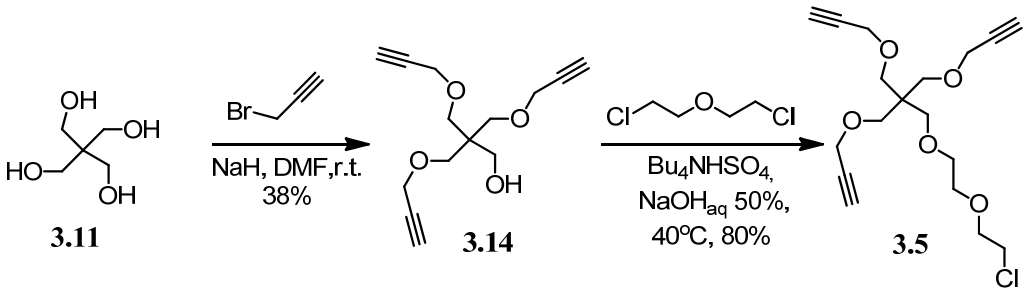
Scheme 3.9 Synthesis of multivalent scaffolds **3.1** – **3.3**

Scaffold **3.4**[22] was prepared from ditosylate derivative **3.13** which was treated with an excess of propargyl alcohol in the presence of potassium carbonate (Scheme 3.10)



Scheme 3.10 Synthesis of multivalent scaffolds **3.4**

The synthesis of **3.5**[21] starts from pentaerytritol **3.11** in which three of the alcohols are functionalized with a propargyl group (**3.14**) and the remaining hydroxyl group is substituted with a linker in the second step (Scheme 3.11).

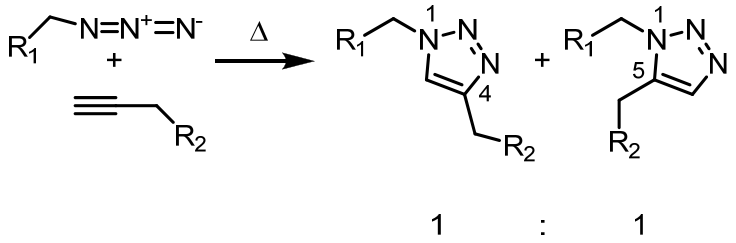


Scheme 3.11 Synthesis of dendronic scaffold **3.5**

3.3.2 Functionalisation, purification

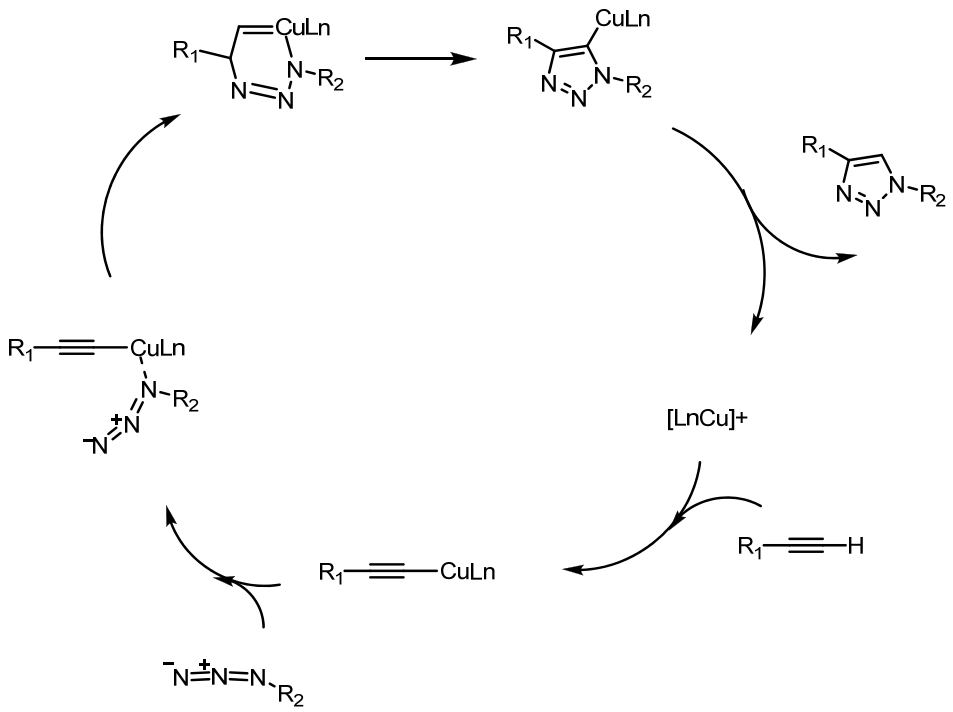
The multivalent scaffolds were functionalised with monovalent ligands **1.7b**, **2.2f** and **1.9**. The used reaction is a 1,3 dipolar cycloaddition which occurs between the triple bond of the scaffold and the azide of the ligand, resulting in a disubstituted triazol ring.

The 1,3-dipolar cycloadition was described by Huisgen in 1968.[28] The reaction required elevated temperature resulting in a mixture of 1,4 and 1,5 subtituted triazols (Scheme 3.12).



Scheme 3.12 Thermally catalyzed 1,3-dipolar cycloadition

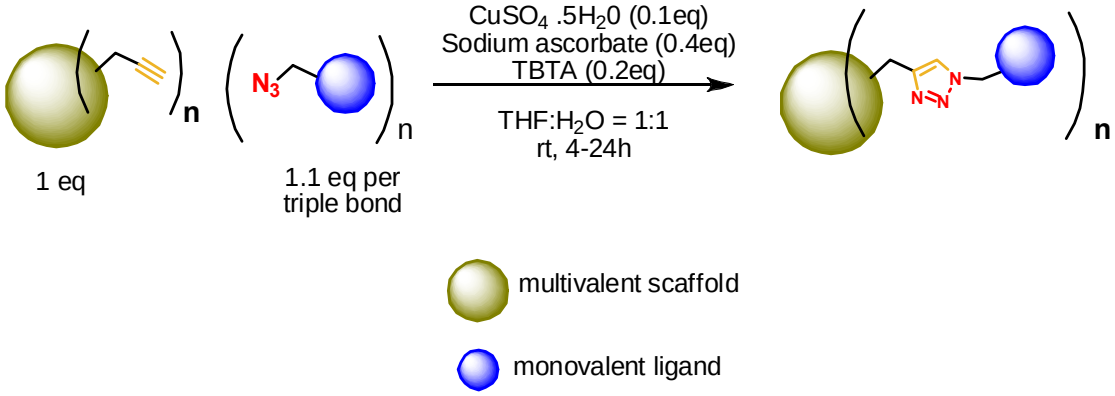
In 2002 the groups of Sharpless[29] and Medal[30] independently reported a regioselective synthesis of 1,4 substituted triazols. The reaction between the alkyne and the azide was mediated by copper(I) salts, which provided regioselectivity and allowed to perform the reaction at room temperture in shorter times. The copper(I) salt creates a salt with the terminal alkyne and at this stage it can coordinate the first nitrogen of the azide group (Scheme 3.13). The proposed mechanism explains the importance of copper(I) in the 1,4 regioselectivity of the reaction as well as the fact that only terminal alkynes can undergo the copper catalyzed transformation.



Scheme 3.13 Copper(I) catalyzed 1,3-dipolar cycloadition

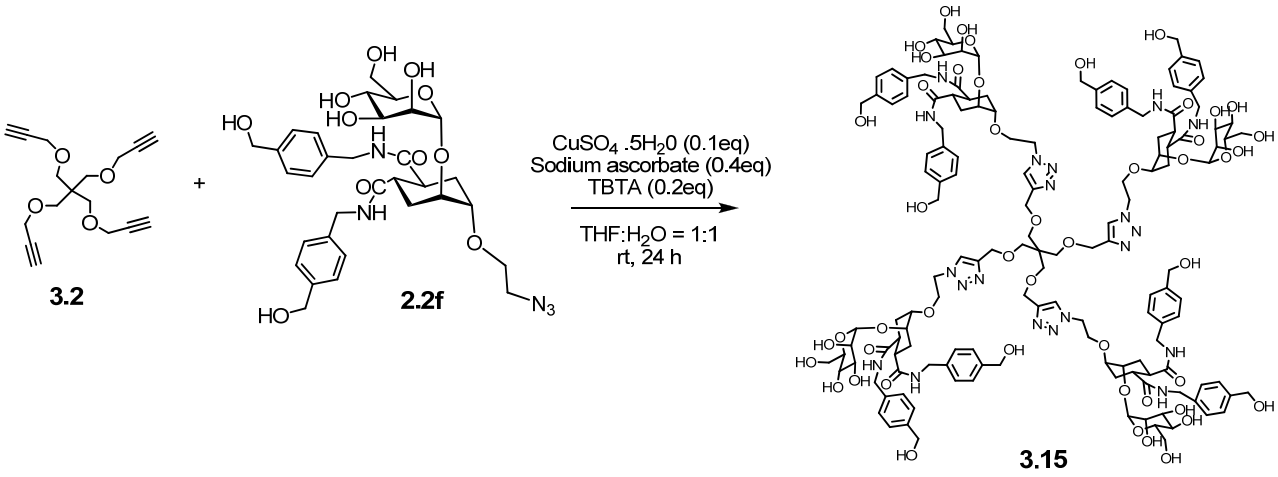
This copper catalyzed cycloaddition tolerates the presence of oxygen, water, and different temperatures or pH, which makes it highly suitable for use in biological systems.[31,32,33] The 1,3 dipolar cycloadition was included in the group of so called "click reactions". The term "click chemistry" is used for those chemical transformations that generate substances quickly and reliably by joining small units together. [34,35,36] This is inspired by the fact that nature also generates substances by joining small modular units.

In order to find the proper conditions to perform the 1,3 dipolar cycloaddition (later know as click reaction) between our substrates, a range of different conditions was screened in the group of Dr. Javier Rojo. The source of copper(I) plays an important role and therefore several salts were tested to perform the click reaction. Finally, a previously described[37] approach using copper(II) sulphate (0.1 equivalent) in combination with sodium ascorbate (0.4 equivalent) was found to be an efficient source of Cu(I). In this system, the ascorbate reduces copper(II) to copper(I) and moreover its excess prevents the possible re-oxidation. In order to stabilize the Cu(I) cation and, once again, prevent its oxidation, tris[(1-benzyl-1H-1,2,3-triazol-4-il)metyl]amine (TBTA) was used as a stabilizing ligand.38 It was found that 1.1 equivalent of azide for 1 equivalent of alkyne is sufficient to achieve full conversion within several hours at room temperature. A mixture of water and THF in 1:1 ratio was used as solvent which efficiently dissolves all the reagents and starting materials (Scheme 3.14).



Scheme 3.14 Reaction conditions used for the copper(I) catalyzed 1,3-dipolar cycloadition (click reaction)

Using the established protocol, several multivalent compounds were synthesized by me and Renato Ribeiro during my stage in Seville (Spain). However, purity issues were observed during the 1H NMR and MASS analysis in some of the prepared compounds. One of the molecules with the most obvious impurity problem was **3.15** prepared from **3.2** and **2.2f** using a click reaction (Scheme 3.15).



Scheme 3.15 Synthesis of **3.15**

The 1H NMR spectra of compound **3.15** showed several peaks in the region 7.9 – 8.1 ppm, which corresponds for the triazol signals (Figure 3.3). However, the structure of the molecule indicates only one singlet.

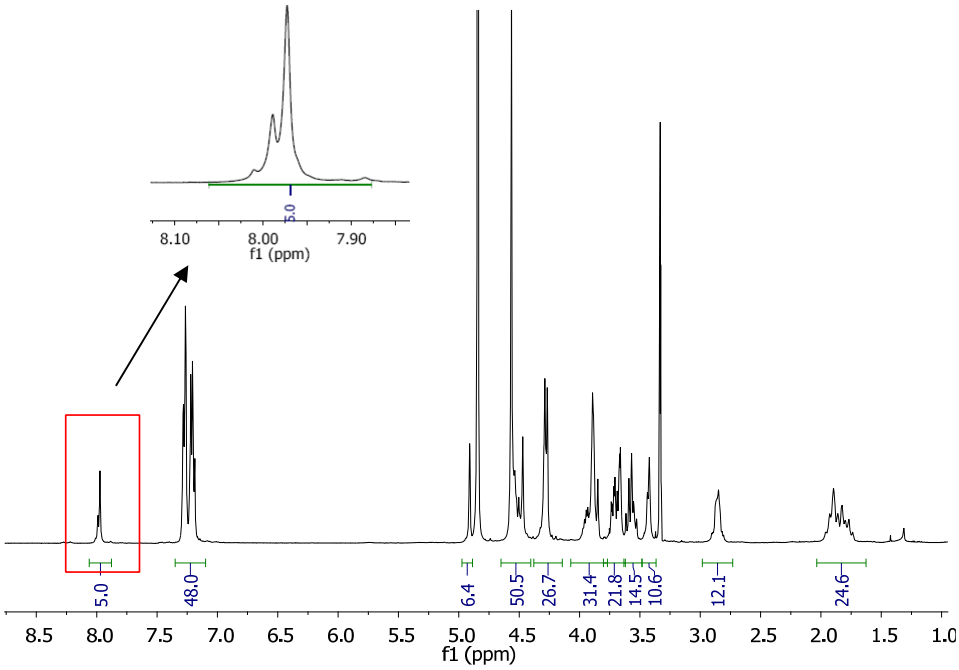
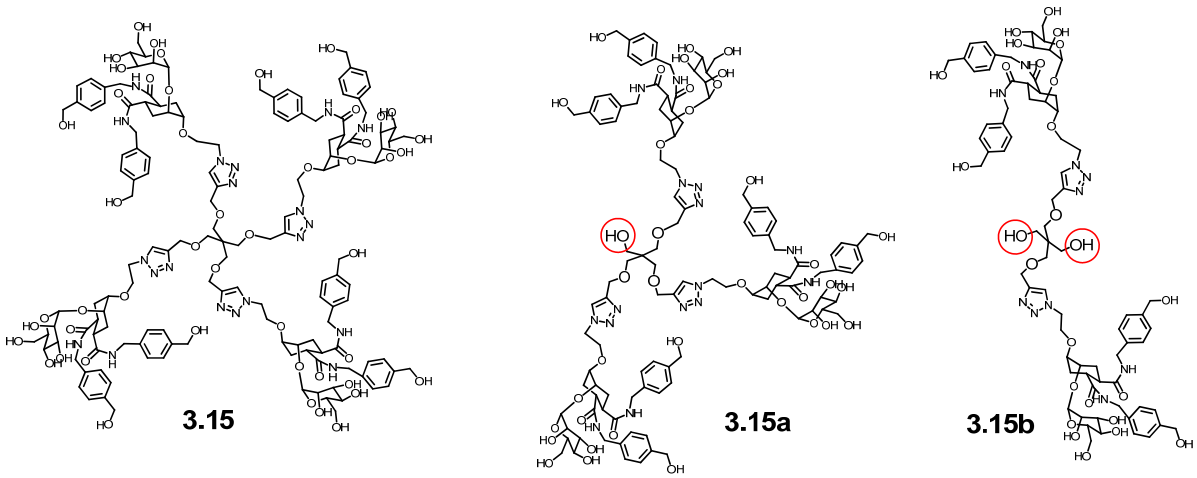


Figure 3.3 1H NMR spectra of the mixture **3.15** with byproducts, (400 MHz, MeOD)

Compound **3.15** was submitted for LC-MASS analysis, which confirmed the presence of several molecules and also gave important information about the molecular weights of components of the mixture (Figure 3.4). The MASS spectrum indicated the presence of molecule **3.15** and of its degradation byproducts **3.15a** and **3.15b** (Scheme 3.16).



$[M+H]^{+}$= 2984.2
$[M+H]^{++}$= 1492.5
$[M+H]^{+++}$= 995.4

$[M+H]^{+}$= 2272.4
$[M+H]^{++}$= 1136.7
$[M+H]^{+++}$= 758.1

$[M+H]^{+}$= 1560.7
$[M+H]^{++}$= 780.8
$[M+H]^{+++}$= 520.9

Scheme 3.16 Structures and molecular weights with single, double and triple charges of product **3.15** and byproducts **3.15a-b**

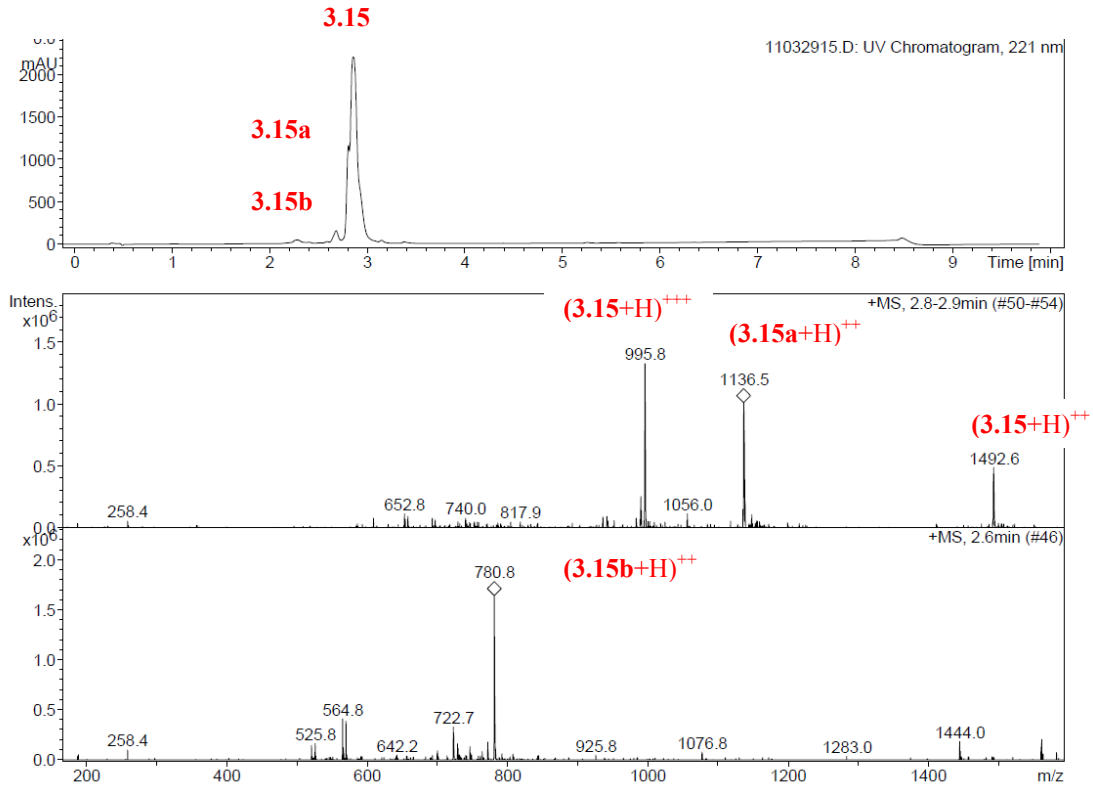


Figure 3.4 LC-MASS analysis of the mixture of **3.15** with impurities [39]

In order to purify the mixture of compounds **3.15** and **3.15a-b**, size exclusion chromatography was performed. Using Sephadex® LH20 matrix and methanol as eluent, after two attempts only partial separation was observed due to the relatively small difference between the molecular weight of **3.15** and **3.15a**. As other possibility to purify the mixture, reverse phase chromatography was used. Using C18 coated TLC plates, water and methanol mixture in 1:1 ratio was found to be an efficient eluent to separate product **3.15** from **3.15a**. However, the reverse phase chromatography (Biotage®, C18 columns, water with gradient of methanol from 0% to 50%) yielded partial separation only with very slow gradient (Figure 3.5).

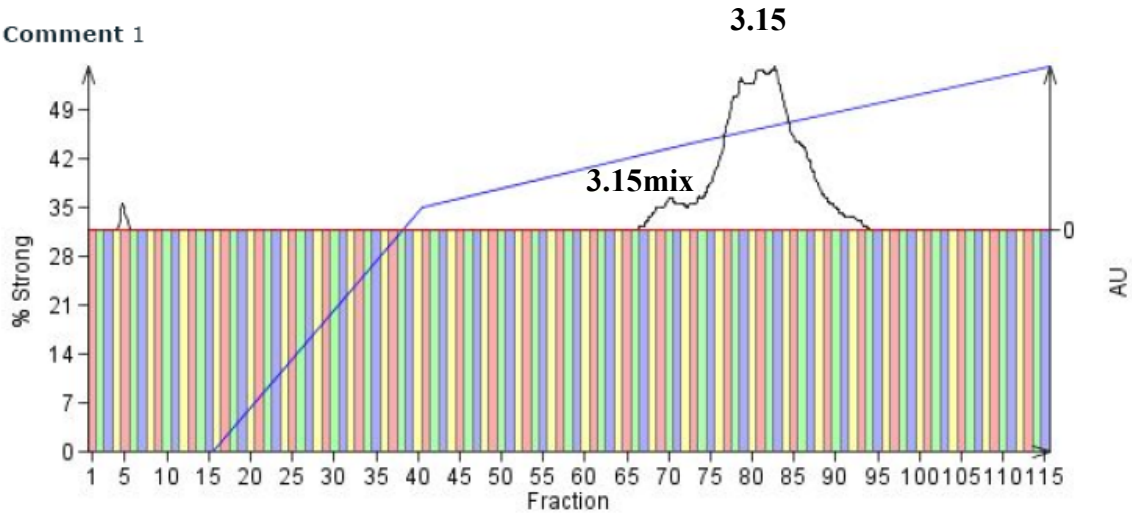


Figure 3.5 Absorption spectra of the fractions coming from the purification of compound **3.15** using C18 reverse phase column. (Collection - 240 nm, Monitor - 210 nm*)*

Nevertheless, the partially separated products were submitted to 1H NMR and MALDI MASS analysis which confirmed the presence of two compounds **3.15** and **3.15a** respectively (Figure 3.6).

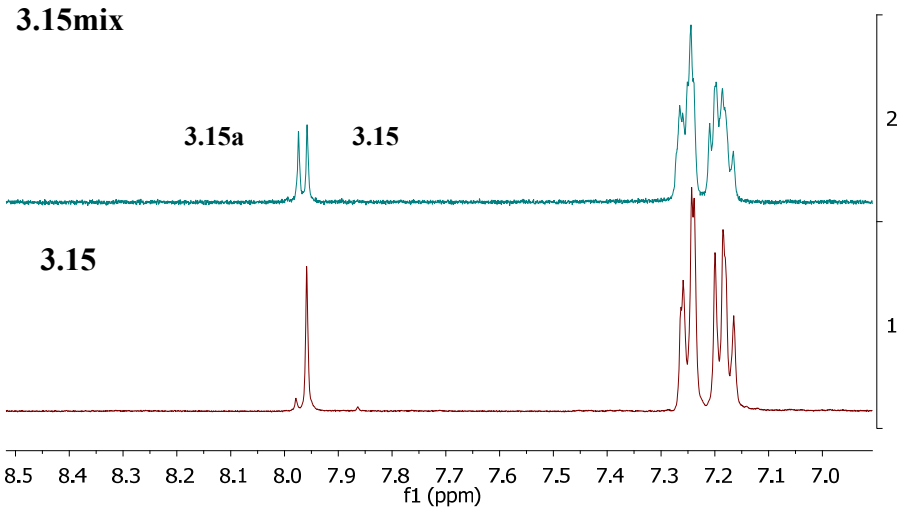


Figure 3.6 1H NMR (MeOD, 400MHz) of fractions collected as **3.15mix** and **3.15** during the purification showed in Figure 3.5

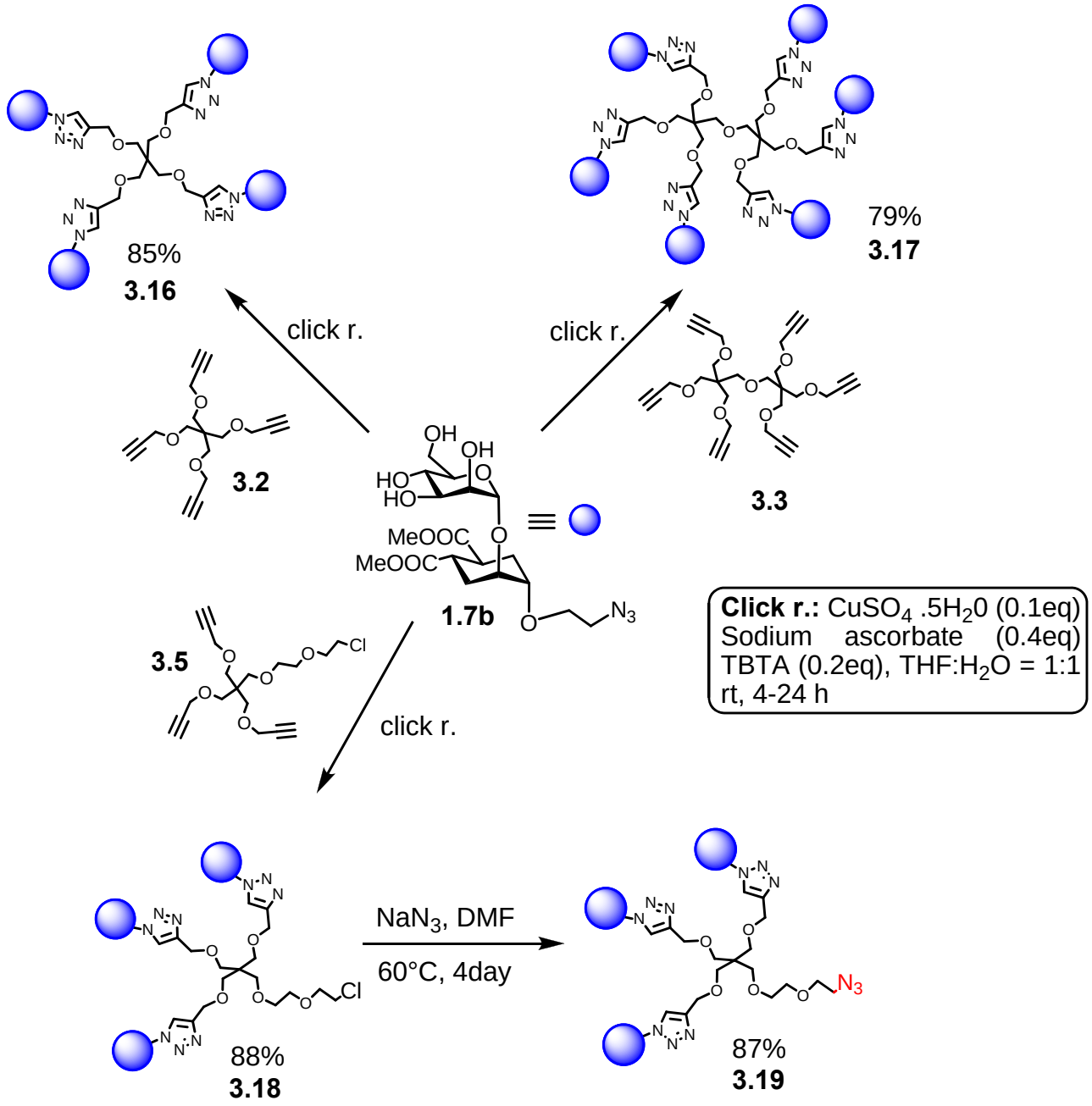
Compounds **3.15** and **3.15a** were not found to be unstable, to click reaction conditions, which suggests that the fragmentation happens with the multivalent scaffold before or during the reactions. It is known that propargyl groups can undergo rearrangements to reactive allenes. This rearrangement can be catalyzed by heat, light or base.[40] Furthermore, it was reported that alkynes and allenes can undergo inter and intramolecular cyclyzations under different conditions.[41] Indeed, it was observed that most of the scaffolds **3.1** - **3.5** decompose in time even at low

temperatures (-20°C). This fact suggests that the scaffolds must be purified immediately before functionalization. With this modification, the synthesis of **3.15** proceeded smoothly and no byproducts were formed. Similarly all other dendrons could be prepared from freshly chromatographed scaffolds in very high purity. It was further observed that often an excess of sodium ascorbate was required to start the reaction or achieve complete conversion. Sodium ascorbate can be easily oxidized after which it can not perform the reduction of copper(II) to copper(I). To avoid this oxidation process, water was degassed in order to remove the dissolved oxygen and THF was freshly distilled to eliminate the presence of peroxides. The reaction was performed under nitrogen atmosphere and in the dark. The reaction mixtures were purified by size exclusion chromatography using Sephadex®-LH20 matrix and methanol as eluent. Since some of the multivalent ligands were studied in biological assays, the potential copper residues had to be removed from in tested compounds. In order to remove the residual copper, the products isolated via size exclusion chromatography were further purified by reverse phase chromatography (C18 columns and water in combination with MeOH or MeCN as eluent) or metal scavengers such as Quadrasil™ MP were used.[42]

3.3.3 Prepared molecules

Although **1.7b** has only moderate activity with DC-SIGN, its relatively easy synthetical accessibility (Chapter 2) and very good water solubility make it a good candidate for the synthesis of a variety of multivalent compounds. Using different multivalent scaffolds with one monovalent ligand can give us important information about the influence of the type of multivalent backbone on the multivalency effect. Therefore, following the general reaction setup (Scheme 3.14), **1.7b** was conjugated with most of the scaffolds mentioned above (Scheme 3.17). In particular, we prepared a series of structures which present 4, 6 or 9 copies of **1.7b**. Additionally, two different hexavalent presentations were generated to examine the efficiency of scaffolds with different lengths.

The click reaction using **1.7b** was initially performed with nuclei **3.2** and **3.3** which resulted in its tetra and hexavalent presentations, **3.16** and **3.17**. Further, ligand **1.7b** was conjugated with **3.5** to afford compound **3.18**, which after treatment with NaN_3 in DMF gave the trivalent dendron **3.19.** This allows the preparation of higher valency compounds by click reaction with alkyne containing scaffolds.



Scheme 3.17 Synthesis of dendrons and dendrimers bearing **1.7b** via click reaction

Compound **3.16** was prepared in 200 mg scale to investigate the scale up possibilities of the click reaction with our substrates. Moreover, **3.16** can be used in different assays and biological studies as a reference compound. 1H NMR and MALDI mass analysis showed good purity (Figure 3.7).

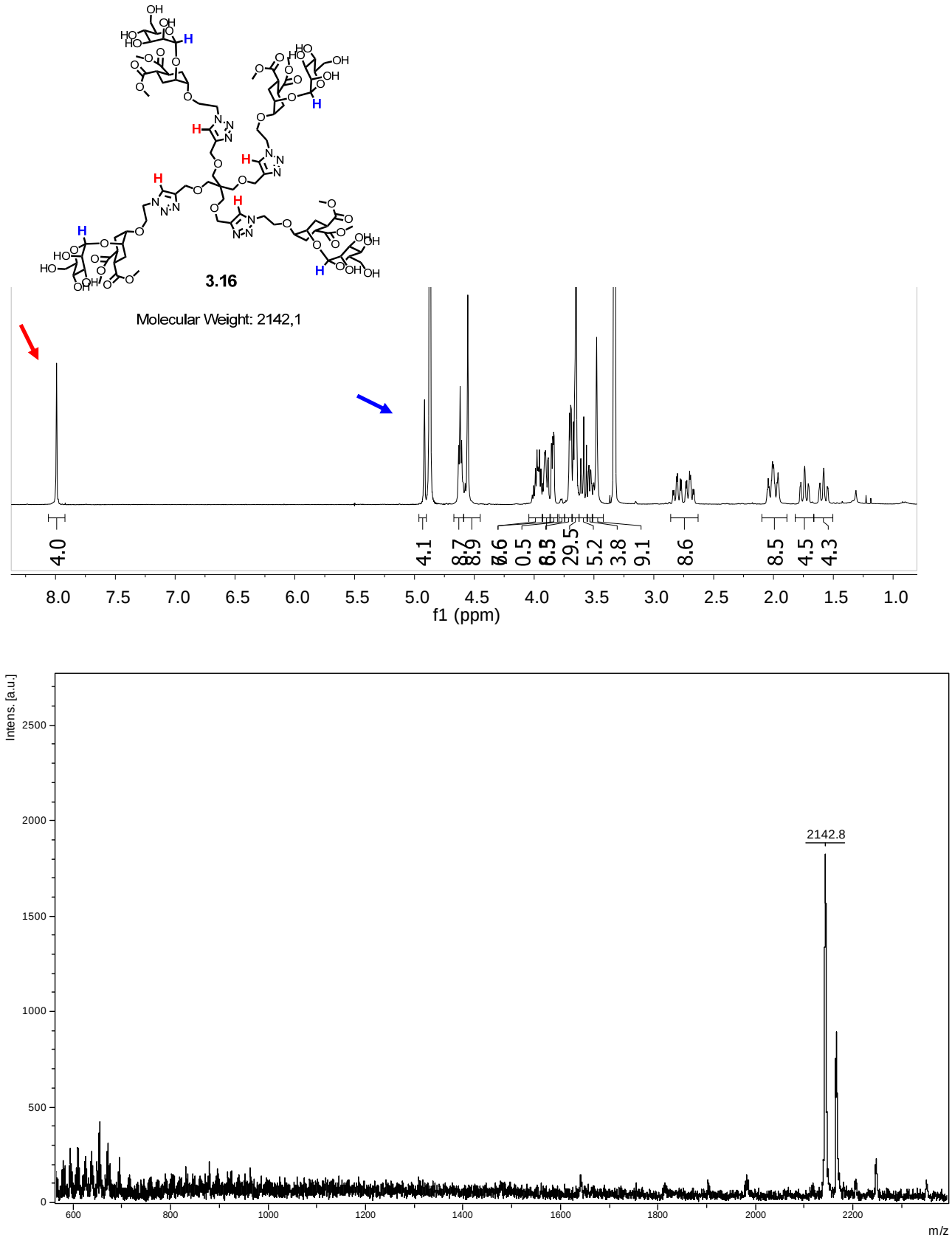
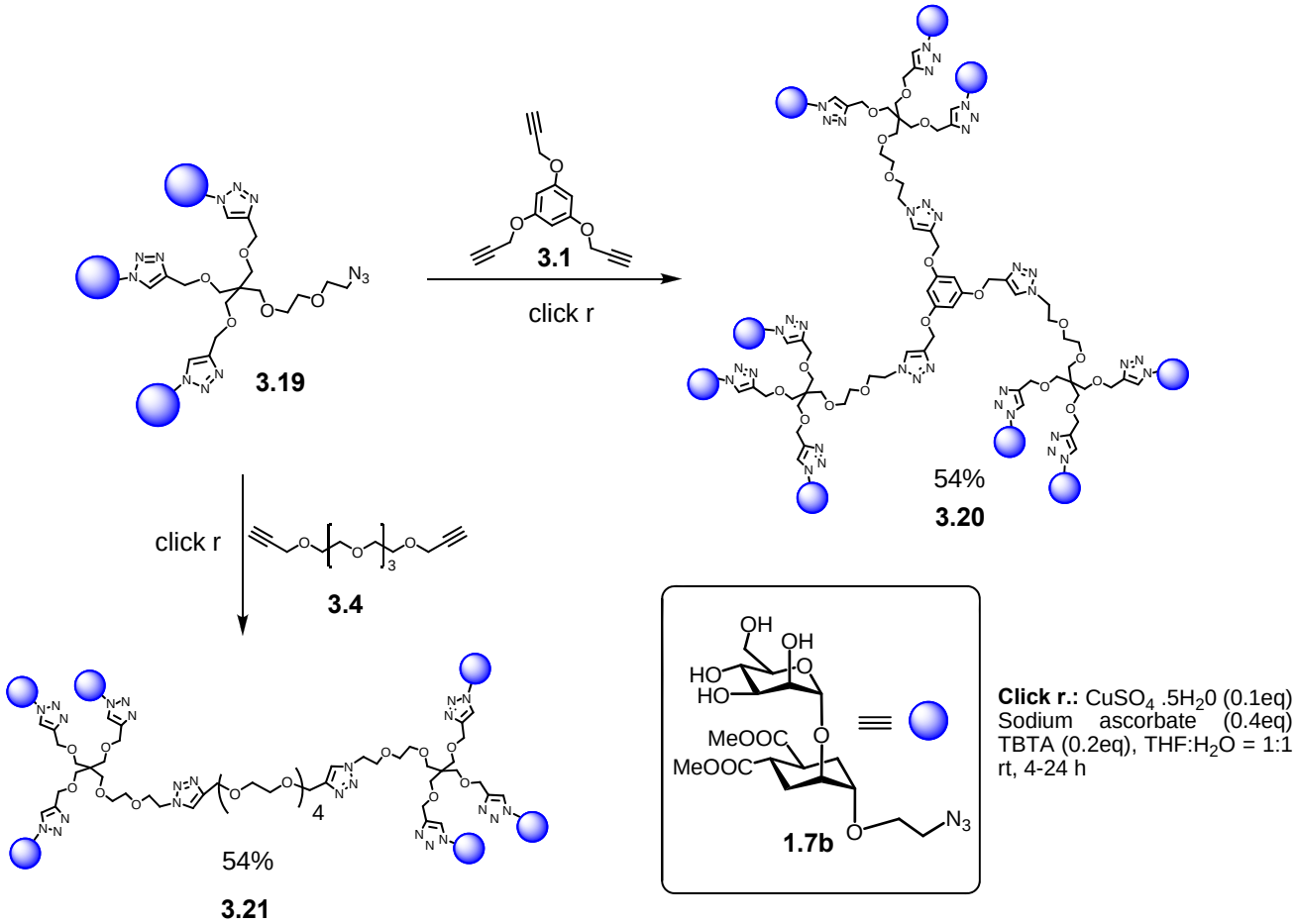


Figure 3.7 ^{1}H NMR (400MHz, CD_3OD) and MALDI mass spectra (matrix: sinapinic acid, solvent H_2O/MeOH) of **3.16**

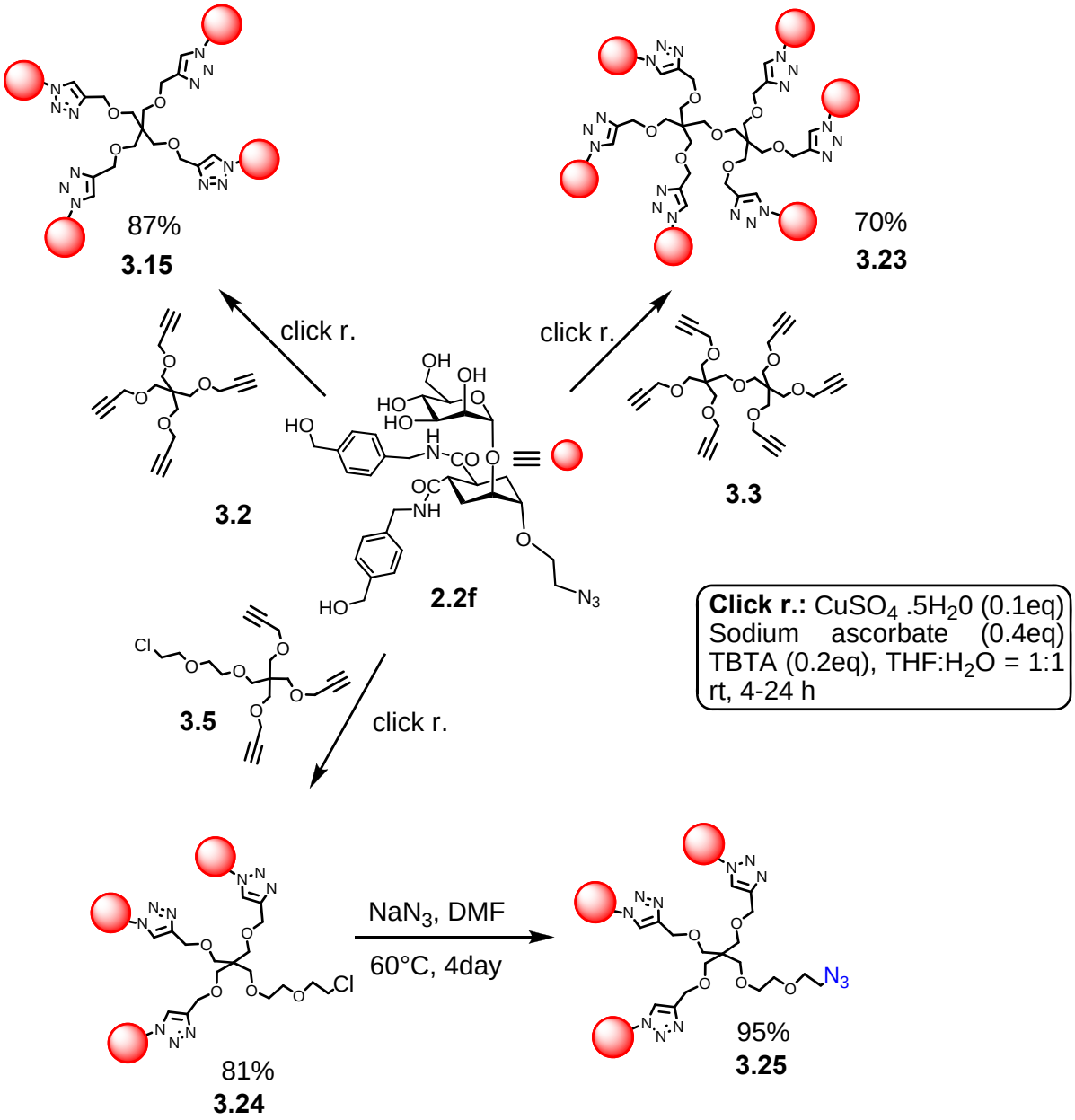
Dendron **3.19** was further conjugated with scaffolds **3.1** and **3.4** which resulted in a nonavalent dendrimer, **3.20,** and a hexavalent structure, **3.21,** with a flexible spacer between the two dendrons (Scheme 3.18).



Scheme 3.18 Synthesis of **3.20** and **3.21** using glycodendron **3.19**

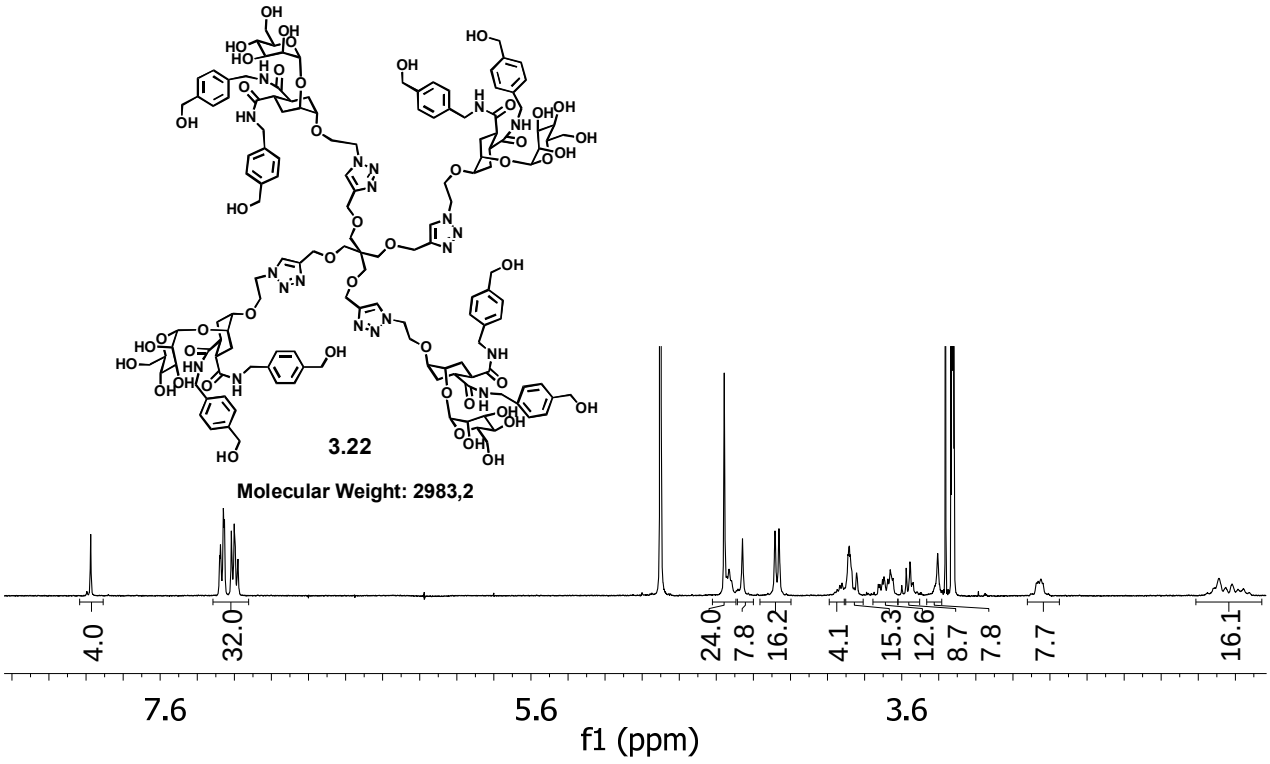
Compounds **3.21** and **3.20** were obtained as mixtures of the desired product as the major component and other byproducts resulting from degradation of the multivalent scaffolds. Both molecules were repeatedly purified using size exclusion chromatography (Sephadex LH20, methanol) and reverse phase chromatography (silica-C18 matrix, methanol:water), however the products were obtained with only approximately 80% purity .

Bisamide **2.2f**, similarly to **1.7b,** was conjugated with the tri, tetra and hexavalent scaffolds to afford the basic multivalent constructs **3.15, 3.23** and **3.24** (Scheme 3.19).



Scheme 3.19 Synthesis of dendrimers (**3.15**, **3.23)** and dendrons (**3.24**, **3.25)** bearing **2.2f** via click reaction

Initially significant purity issues were observed with the prepared molecules, but when the click reactions were repeated using the optimized protocol described above the desired compounds were obtained with good purity (Figure 3.8).



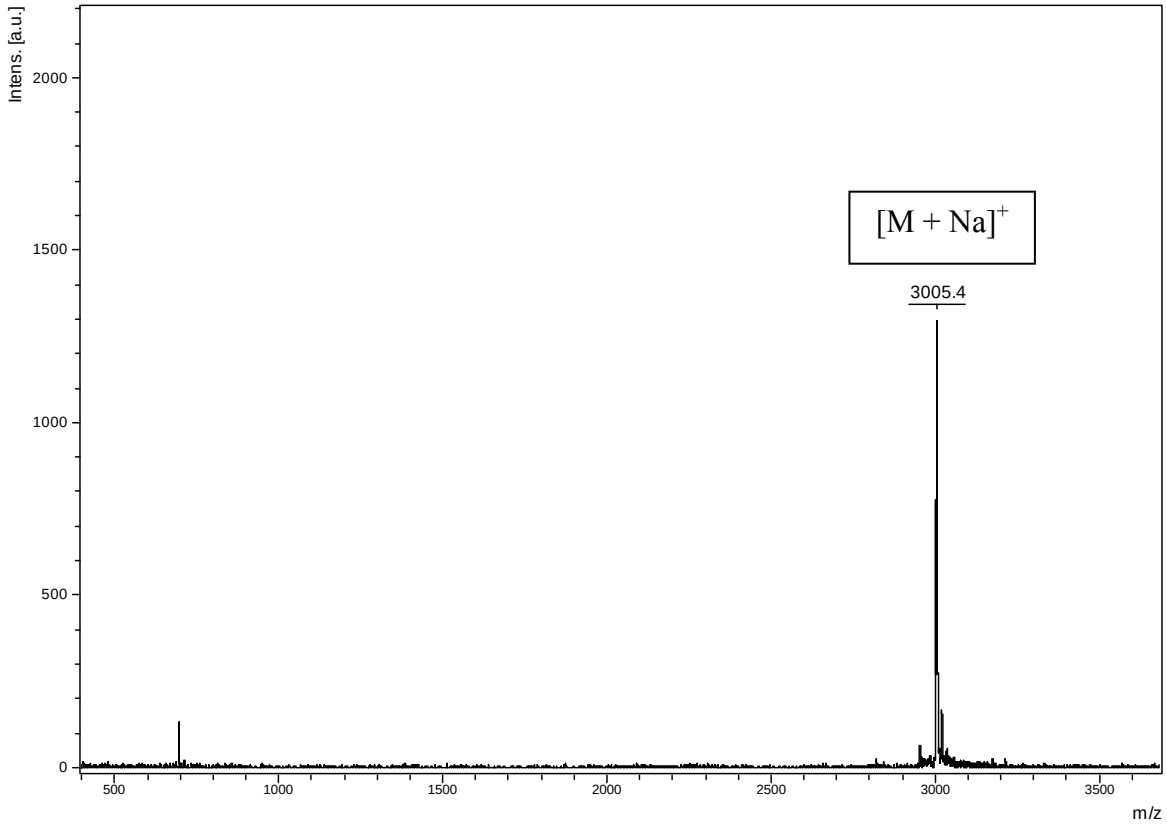
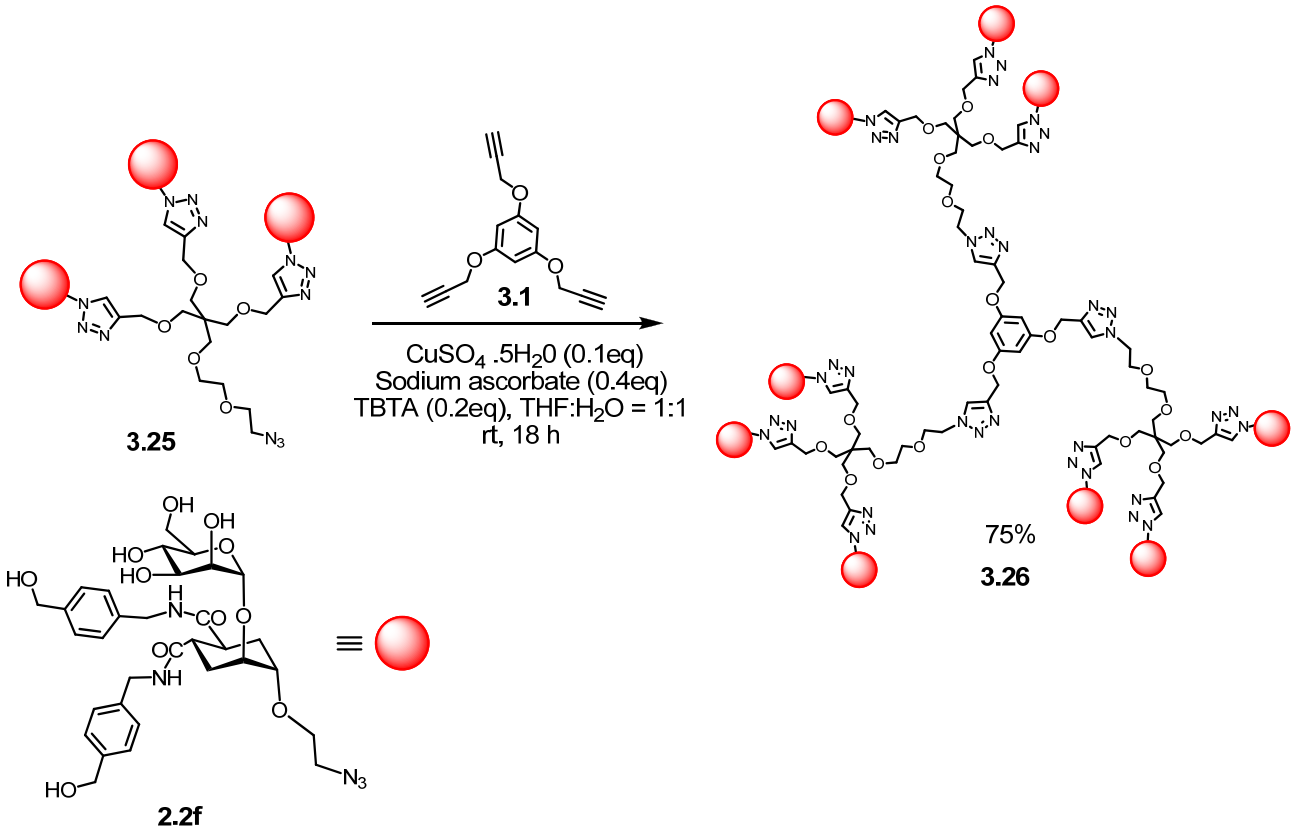


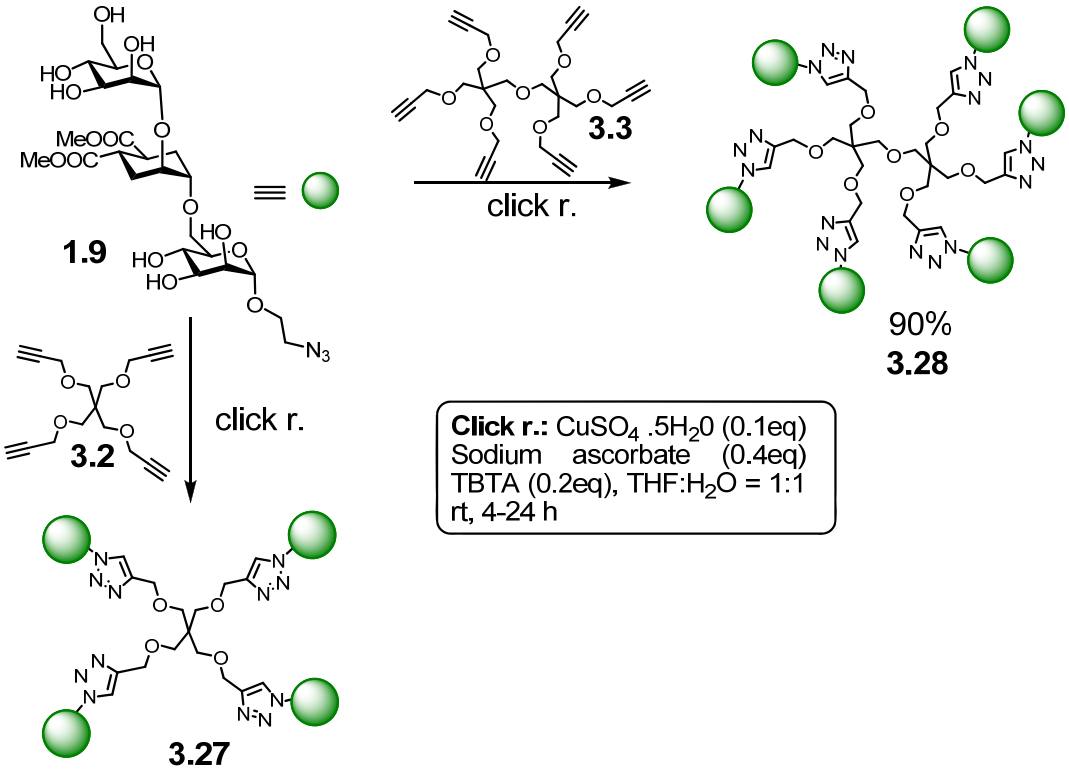
Figure 3.8 ^{1}H NMR (400Mhz, MeOD) and MALDI mass spectra (matrix: α-cyano-4-hydroxy-cinnamic acid, solvent: MeOH) of **3.15**

Dendron **3.25** was connected via click reaction to nucleus **3.1** in order to obtain multivalent ligand **3.26** with nine copies of **2.2f** (Scheme 3.20). The product was obtained in relatively good purity but unfortunately significant solubility problems were observed. It was found that in the case of multivalent ligands bearing **2.2f** solubility in water decreases with the growing valency. Probably the high number of aromatic residues (benzyls of **2.2f**, triazols, and central benzene core) give a rather lipophylic profile to the molecule, moreover, π-π stacking interactions between the aromatic groups can have a negative effect on the solubility in general. No solubility problems were observed with tetravalent **3.15,** but hexavalent construct **3.23** already showed signs of lower water solubility. In the case of **3.26** with nine **2.2f** residues, the solubility was at the limit of approximately 2mg/ml, corresponding to a concentration of 0.27 μM. This can be a crucial problem during the determination of IC50 since the experiments are performed in water media, and leads to the conclusion that six copies of **2.2f** within a multivalent structure is on the limit in terms of solubility for these constructs.



Scheme 3.20 Synthesis of nonavalent dendrimer **3.26** using dendron **3.25**

Finally, the most potent monovalent ligand prepared in our laboratory, the pseudotrisaccharide **1.9**, was conjugated with tetra and hexavalent structures to obtain multivalent ligands **3.27** and **3.28** (Scheme 3.21).

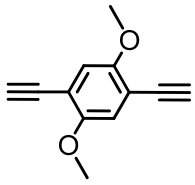


Scheme 3.21 Synthesis of dendrimers **3.27** and **3.28** bearing **1.9** via click reaction

3.3.4 Molecular rods

In the first part of this chapter a general goal to prepare multivalent structures containing a rigid rod-like core was described. The two terminals of the central rod would be decorated with flexible dendrons bearing DC-SIGN ligands. The aim of this kind of structures is to bind simultaneously two binding sites within one CRD (Figure 3.2 and Scheme 3.8). As it was stated in the previous section, with increasing valency the solubility of multivalent compounds starts to be a significant issue. Therefore, rather than preparing polyvalent compounds with high valency, the investigation focused on multivalent scaffolds that can control the position and orientation of the monovalent ligands and thus achieve efficient binding with lower valency.

The first and simplest molecule **3.7a** (Figure 3.9) which could act as a rod was previously described and prepared for different purpouses[43].

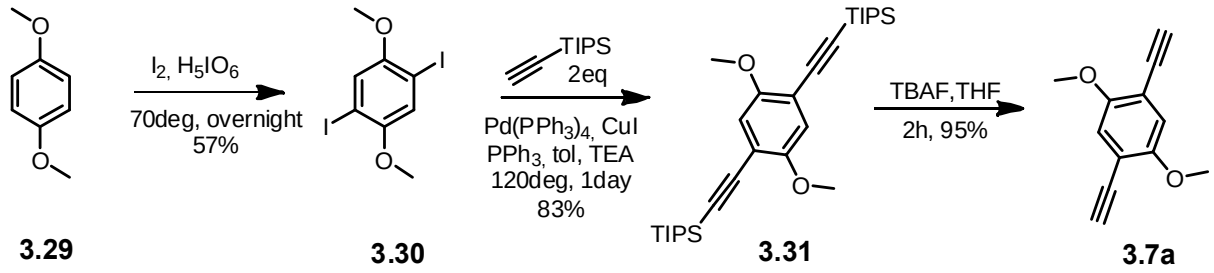


3.7a

Figure 3.9 Structure of rod **3.7a**

Although its length may not be sufficient to achieve the desired multiple binding, its structural simplicity can be useful to optimize the functionalization step with dendrons such as **3.25** and to give us information about the properties of multivalent rod-like structures. Further, ligands built on rod **3.7a** can be a good comparison with molecules which are long enough to reach two binding sites.

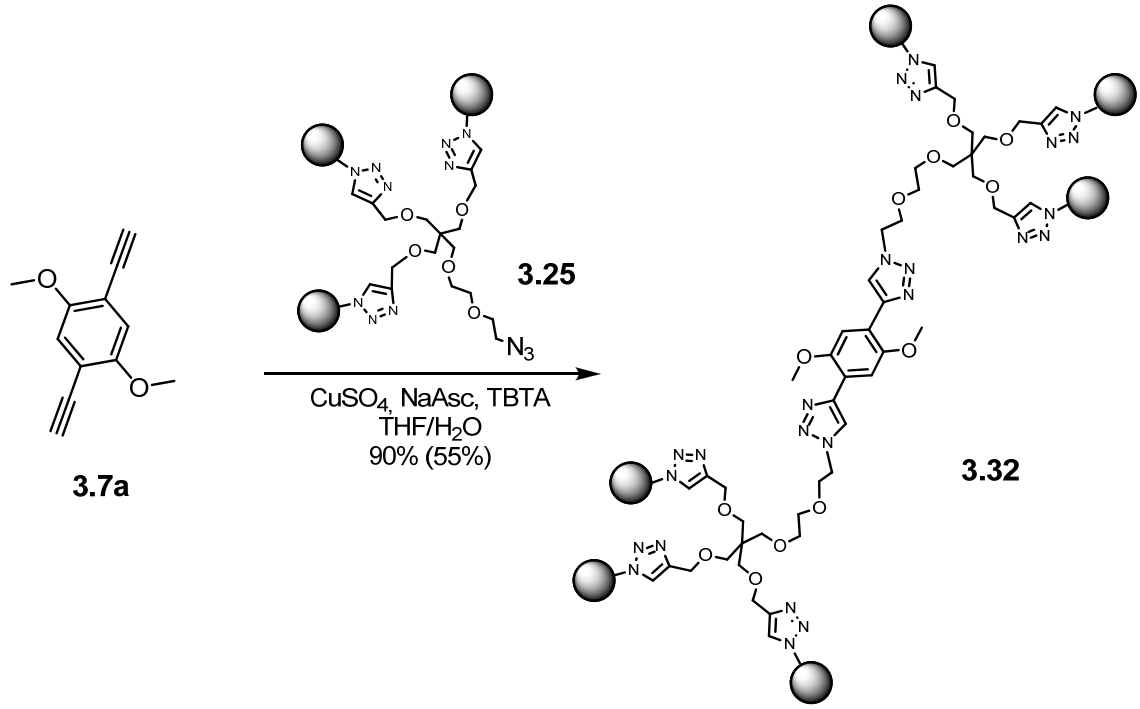
The synthesis of **3.7a** is relatively simple: it can be prepared in three steps (Scheme 3.22). In the first reaction 1,4-dimethoxy benzene **3.29** is functionalized in positions 2 and 5 with two iodide moieties, which are substituted in the following step with triisopropylsilylacetylene (TIPS-acetylene) via Sonogashira reaction, affording compound **3.31**. The TIPS groups are cleaved with TBAF to obtain **3.7a** in high yield.



3.29 **3.30** **3.31** **3.7a**

Scheme 3.22 Synthesis of **3.7a**

In order to prepare the first multivalent DC-SIGN ligand with a rigid spacer, compound **3.7a** was functionalized with dendron **3.25** bearing three copies of monovalent ligand **2.2f** (Scheme 3.23). The click reaction was performed using the conditions described in the previous section and, after purification by size exclusion chromatography, the product was isolated in high yield (90%, Scheme 3.22). However after the subsequent purification by reverse phase chromatography the yield dropped to 55%. It was later found that **3.32** has solubility issues in water, which could explain the loss of product during the purification using reverse phase.



Scheme 3.23 Synthesis of **3.32** via click reaction between **3.7a** and **3.25**

In order to improve the solubility of the final molecule compound **3.7a** was modified. The methoxy groups were substituted with short polyethyleneglycol chain (PEG), which should increase the polarity of the molecule and enhance the solubility in water.

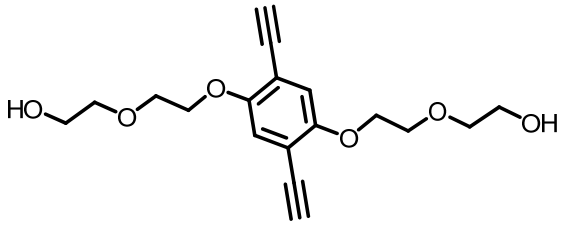
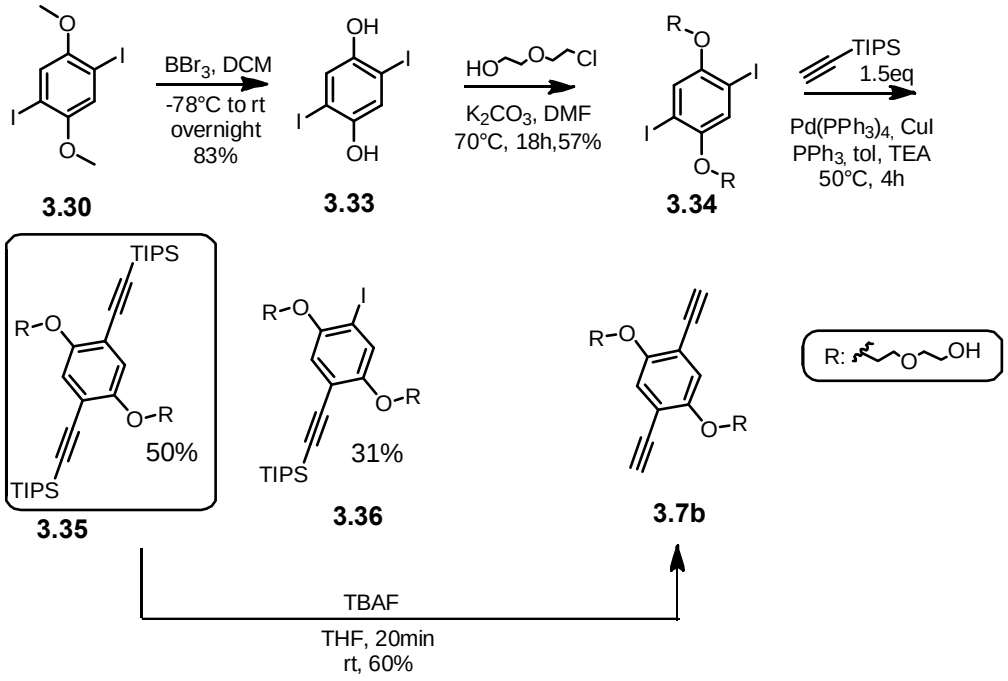


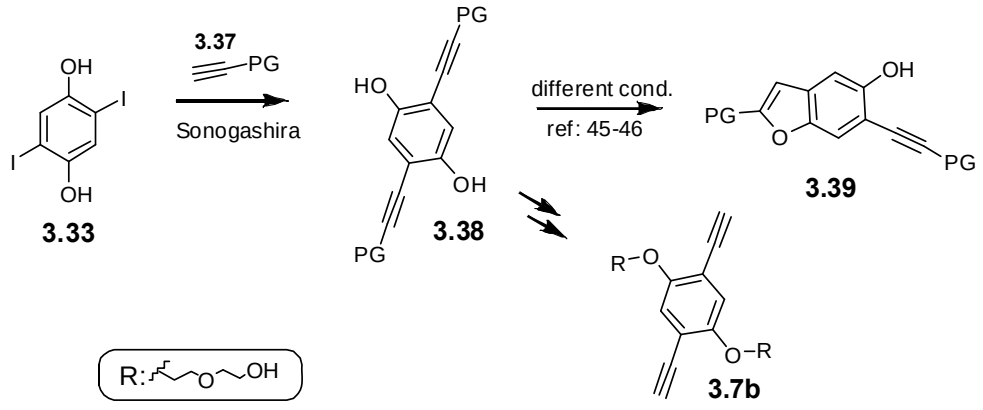
Figure 3.10 Structure of **3.7b**

The synthesis of **3.7b** starts form the previously obtained **3.30** (Scheme 3.22) which is treated with BBr_3 at low temperature in order to cleave the methyl ethers and obtain the bis-phenol **3.33** (Scheme 3.24). In the following step, short PEG chains are introduced by treatment of **3.33** with 2-(2-chloroethoxy)ethanol in the presence of K_2CO_3. With product **3.34** Sonogashira coupling was performed using 1.5 eq. of TIPS-acetylene, which resulted in the mono and disubstituted products **3.35** and **3.36**. **3.35** was deprotected using TBAF to obtain the final product **3.7b** (Scheme 3.23), whereas the monosubstituted compound **3.36** was used during the synthesis of longer rods.



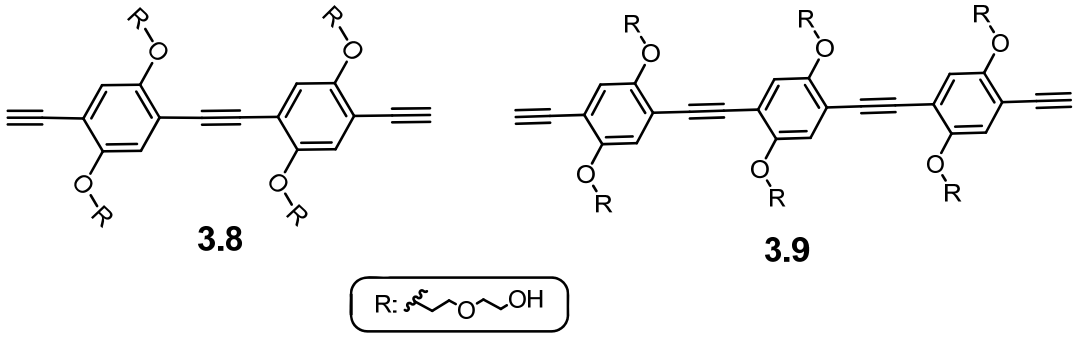
Scheme 3.24 Synthesis **of 3.7b**

Another reaction path to obtain product **3.7b** could be functionalization of **3.33** with an alkyne derivative **3.37** which would result in **3.38** that could be further elaborated to obtain the desired rod (Scheme 3.25). However, it was previously described that structures as **3.38** undergo intramolecular cyclisations to benzofuranes (**3.39**). This cyclization can be promoted by various reaction conditions, as mild bases,[44] palladium/copper catalytic systems,[45] TBAF[46] and UV irradiation [47] Therefore this reaction route was not investigated.



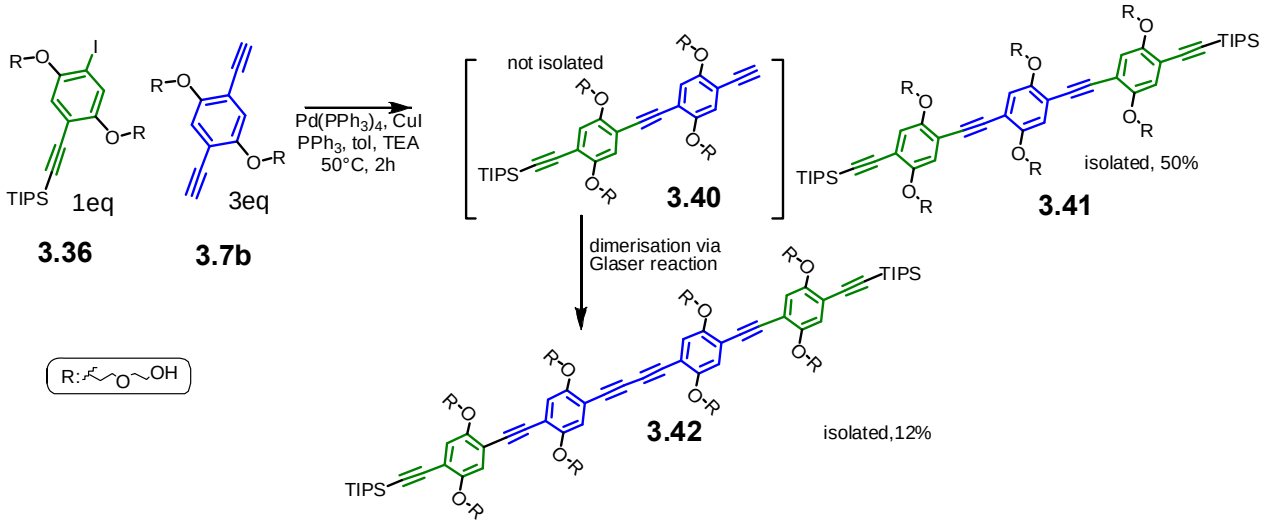
Scheme 3.25 Alternative reaction route for the synthesis of **3.7b** and the possible cyclysation of its intermediate **3.38**

In order to obtain rods with longer structures, another two molecules have been proposed. The first one (**3.8**) contains two aromatic rings connected via a triple bond, whereas the second molecule (**3.9**) consists of three aromatic rings also connected with triple bonds (Scheme 3.26).



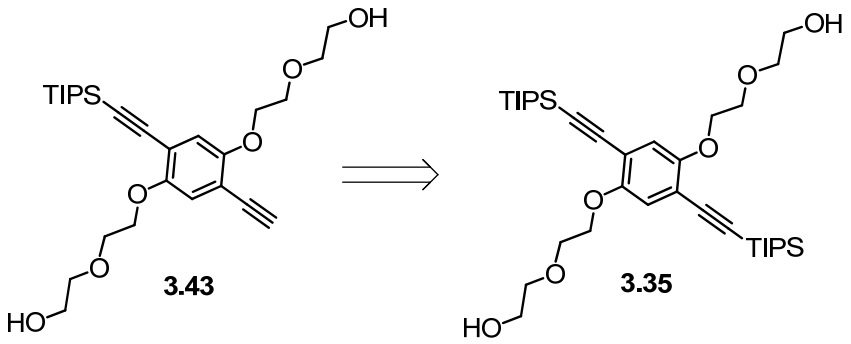
Scheme 3.26 Structure of rod-like scaffolds **3.8** and **3.9**

The first attempt to prepare the desired molecules used a Sonogashira coupling between the previously prepared molecules **3.36** and **3.7b** (Scheme 3.27). This reaction was performed several times using different conditions but in most cases a complex mixture of products was obtained. The purification of the products was demanding, however two compounds were isolated.



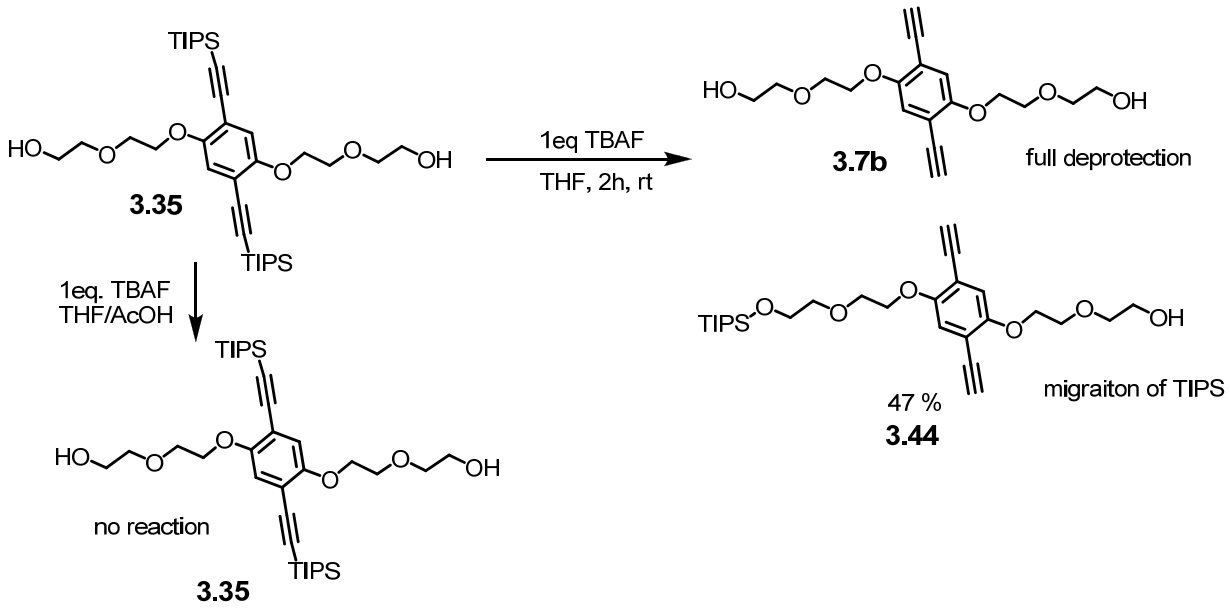
Scheme 3.27 Synthesis of the desired molecule **3.40** and **3.41** using Sonogashira reaction between **3.36** and **3.7b,** and the subsequent homo-coupling of **3.40** (Glaser reaction)

Product **3.41** is a precursor for the desired rod **3.9**, however **3.42** is the result of dimerisation of the desired molecule **3.40** via copper(I) catalyzed Glaser reaction.[48,49,31] In order to avoid the homo-coupling of **3.40**, the monoprotected bis-alkyne **3.43** would be required (Scheme 3.28). This could be, in principle, obtained by monodeprotection of **3.35**.



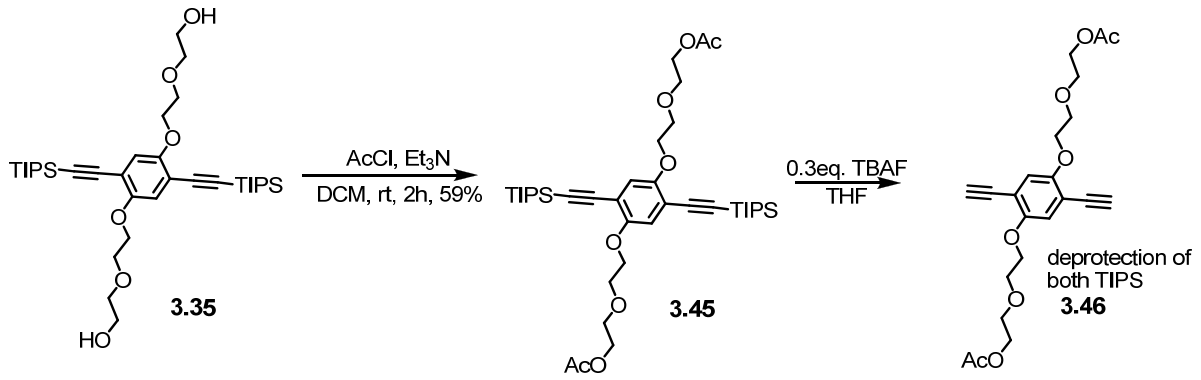
Scheme 3.28 structure of **3.43** and its possible precursor **3.35**

The attempts to deprotect only one of the triple bonds in **3.35** using one equivalent of TBAF resulted either in double deprotection (to **3.7b**, Scheme 3.29) or in the migration of the TIPS group to the oxygen atom (**3.44**, Scheme 3.29). This suggested that the cleavage can be catalyzed by the basic nature of TBAF, therefore THF in combination with acetic acid was used as solvent to prevent the deprotection of the second TIPS group.



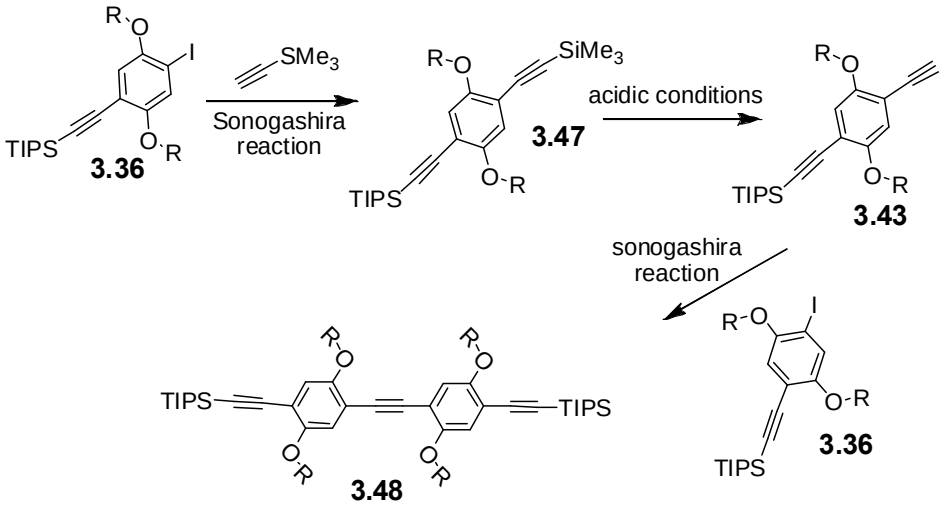
Scheme 3.29 Attempts to selectively deprotect only one of the TIPS groups in **3.35**

Interestingly, it was observed that in acidic media none of the silyl groups was cleaved using TBAF. To prevent the migration of the sylyl groups, the alcohols were protected with acetyl moieties (**3.45)**, however the subsequent reaction resulted again in full deprotection even when only 0.3 equivalent of TBAF was used (Scheme 3.30).



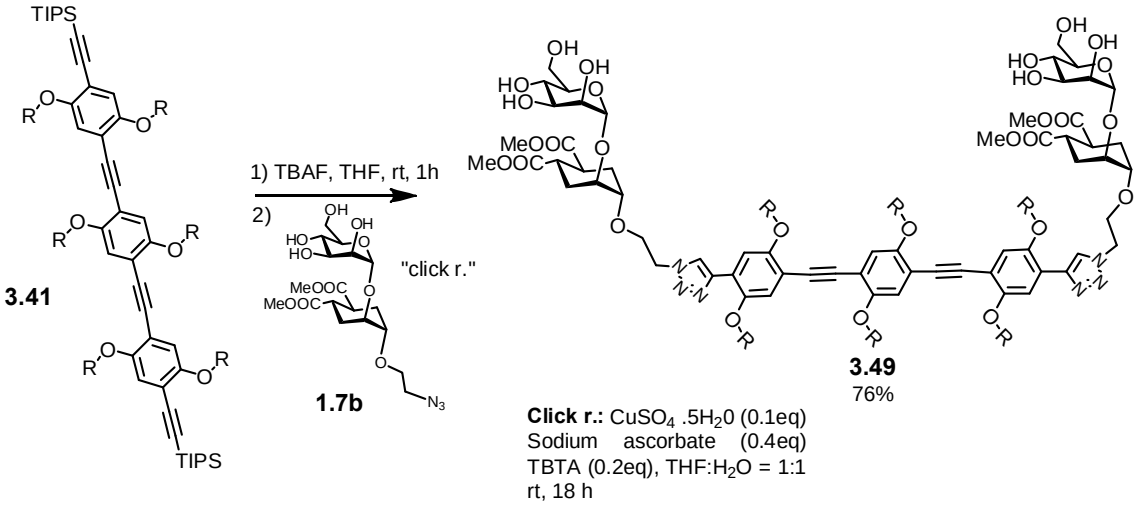
Scheme 3.30 Protection of the free OH groups in **3.35** in order to prevent the migration of TIPS and the subsequent attempt to deprotect only one of the TIPS groups in **3.45**

The synthesis of **3.8** still remains a target of our study. One of the options to achieve our goal is to prepare molecule **3.47** in which the alkynes are protected with two different protecting groups. The diverse nature of silyl groups could facilitate the selective deprotection (Scheme 3.31).



Scheme 3.31 A possible reaction path to obtain the precursor of **3.8,** compound **3.48**

Nevertheless, in order to obtain a DC-SIGN ligand with a rod-like structure our study focused on compound **3.9** and its precursor **3.41**. The first functionalisation of **3.9** was performed with pseudodisacharide **1.7b** used as a model system (Scheme 3.32). In the first step compound **3.41** was deprotected with TBAF, and the product was not isolated from the reaction mixture; rather ligand **1.7b** was added and the click reaction was performed in one pot.



Scheme 3.32 Synthesis of **3.49**

The product was isolated with good purity and yield and no chemoselectivity problems concerning the internal triple bonds were observed (Figure 3.11).

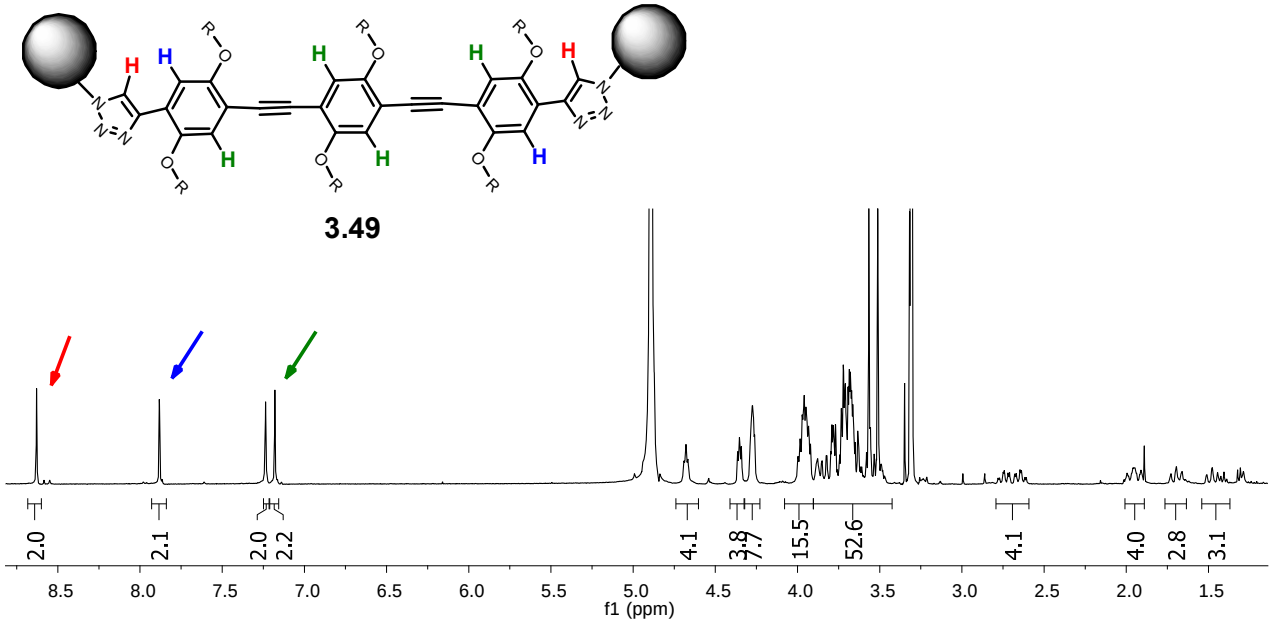
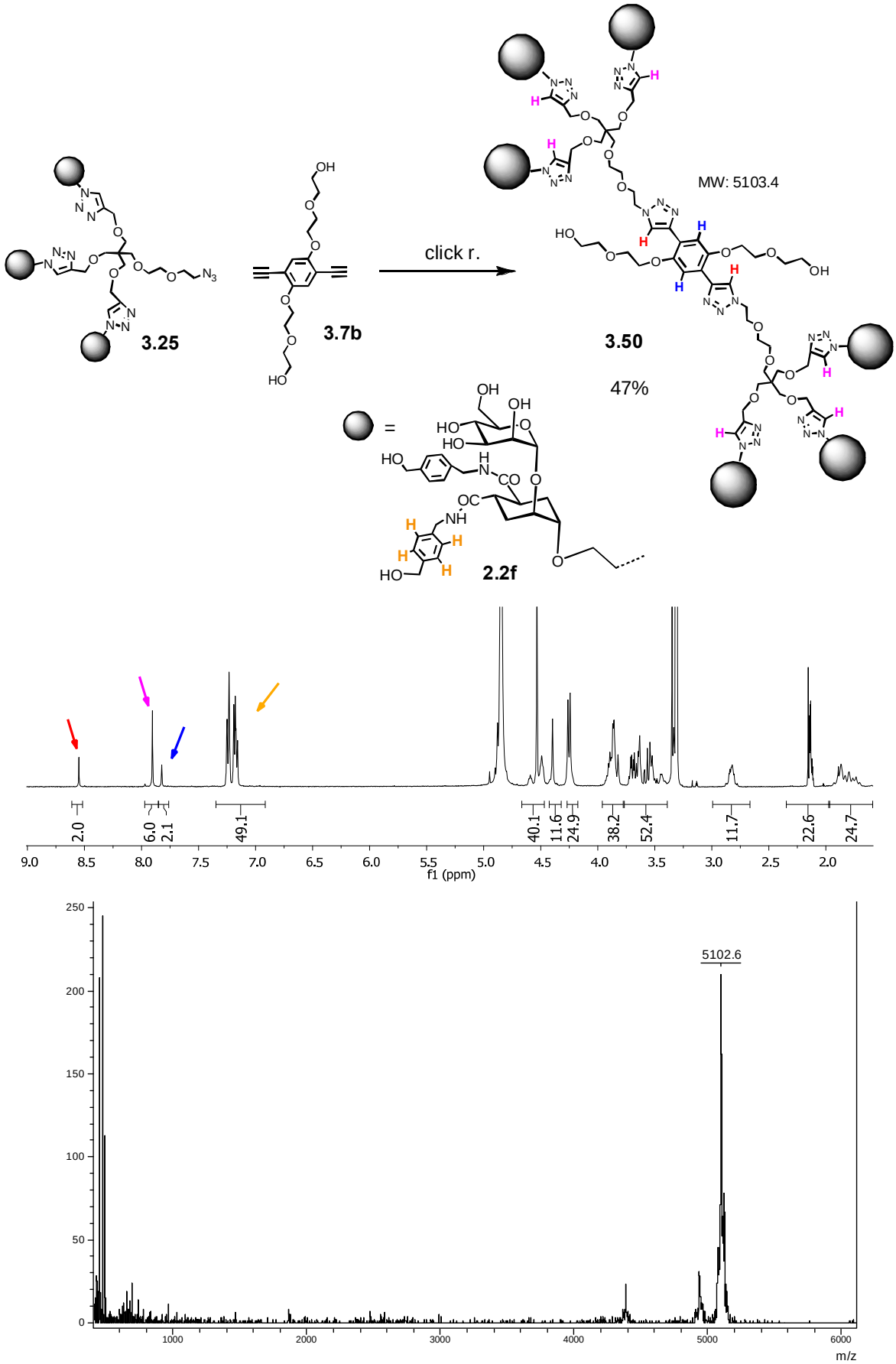


Figure 3.11 Structure and 1H NMR (CD_3OD, 400MHz) of **3.49** with the assignement of aromatical protons

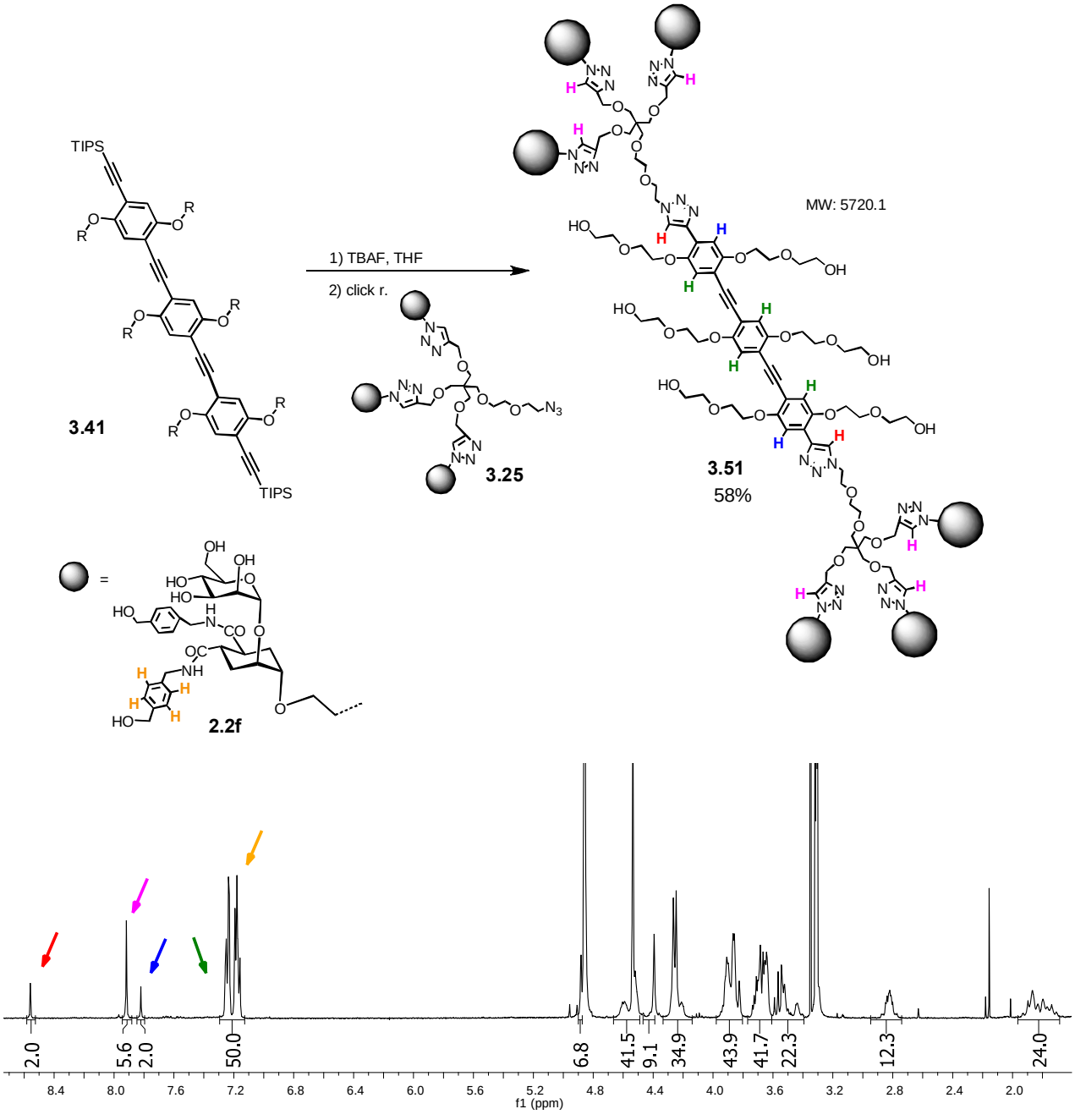
The two final reactions, in order to obtain our target molecules, were performed between dendron **3.25** and rod–like structures **3.7b** and **3.9** (Scheme 3.33 and 3.34). The synthesis of **3.50**

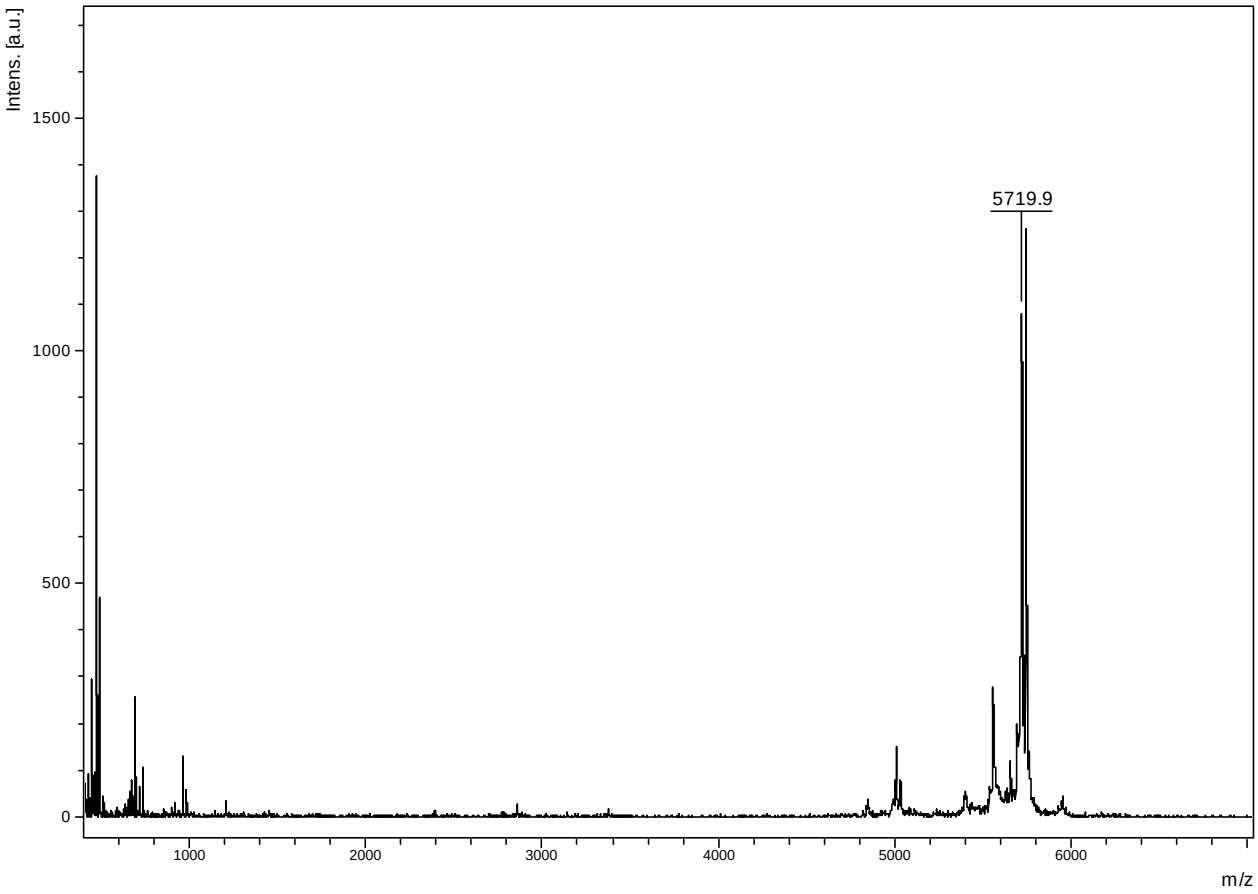
was achieved by clicking **3.25** on **3.7b** under the click conditions. The product was isolated using size exclusion chromatography followed by reverse phase chromatography (Scheme 3.33).



Scheme 3.33 Synthesis, 1H NMR (400MHz, CD_3OD) and MALDI mass (matrix: sinapinic acid, solvent: MeOH) spectra of **3.50**

Similarly to the reaction shown in Scheme **3.32**, for the synthesis of **3.51** the TIPS groups of **3.41** were cleaved in the first step using TBAF and subsequently the click reaction was performed (one pot). Compounds **3.50** and **3.51**were obtained in good purity and moderate yields.





Scheme 3.34 Synthesis, 1H NMR (400MHz, CD_3OD) and MALDI mass (matrix: sinapinic acid, solvent: MeOH) spectra of **3.51**

Although the solubility of **3.50.** was improved in comparison with **3.32** it still remains a rather significant problem.

3.4 Activity determination of multivalent structures with DC-SIGN

3.4.1 SPR

Similarly to monovalent ligands described in the second chapter, the multivalent glycodendrimers described above were tested initially using SPR. The measurements were performed in the group of professor Franck Fieschi[50] by a PhD student, Ieva Sutkeviciute. Full IC50 curve was measured for each compound and the experimental setup was identical to the one used for the monovalent ligands showed in the previous chapter. A competition experiment was used, and the ligands were tested for their ability to inhibit binding of DC-SIGN to Man-BSA which is immobilized on the surface of the chip. The multivalent compounds were tested in two different campaigns, but some of them were tested in both campaigns as standards in order to obtain more accurate comparison between the examined ligands.

In the following figure, the structures and activities of **1.7b** and of the multivalent constructs derived are shown.

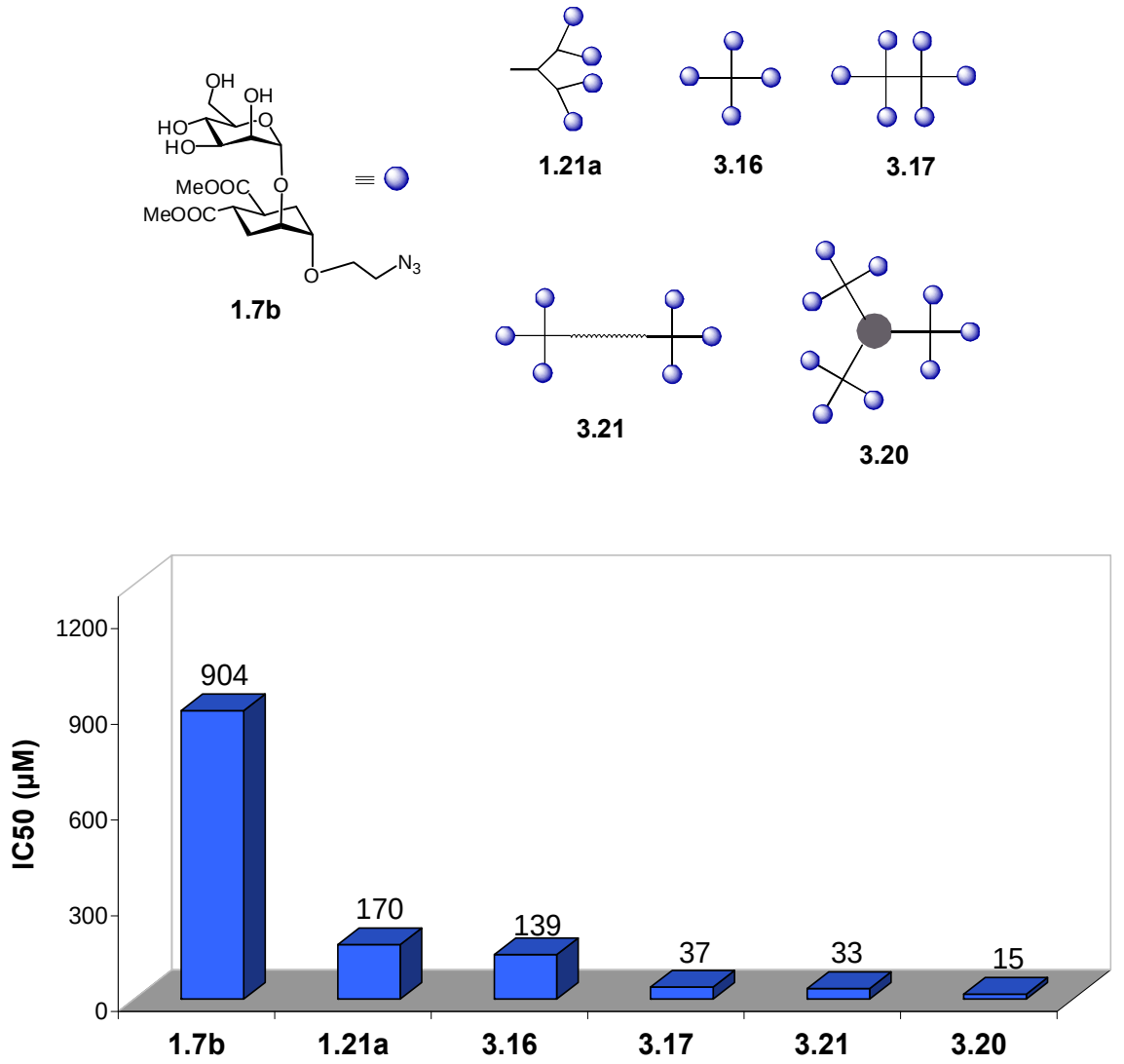
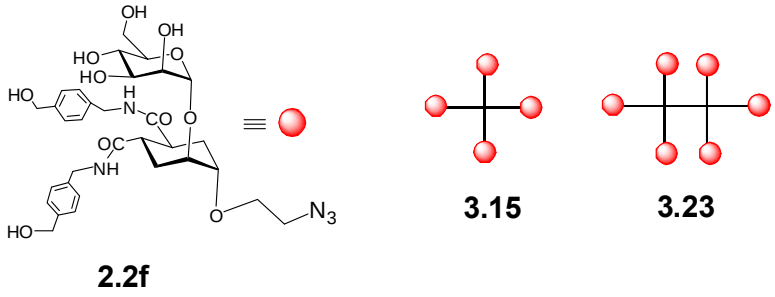


Figure 3.12 Schematic structures and IC50 values of **1.7b**, glycodendron **1.21a** (Boltorn type) and glycodendrimers **3.16**, **3.17**, **3.21** and **3.20** (derived from erythritol) measured by SPR

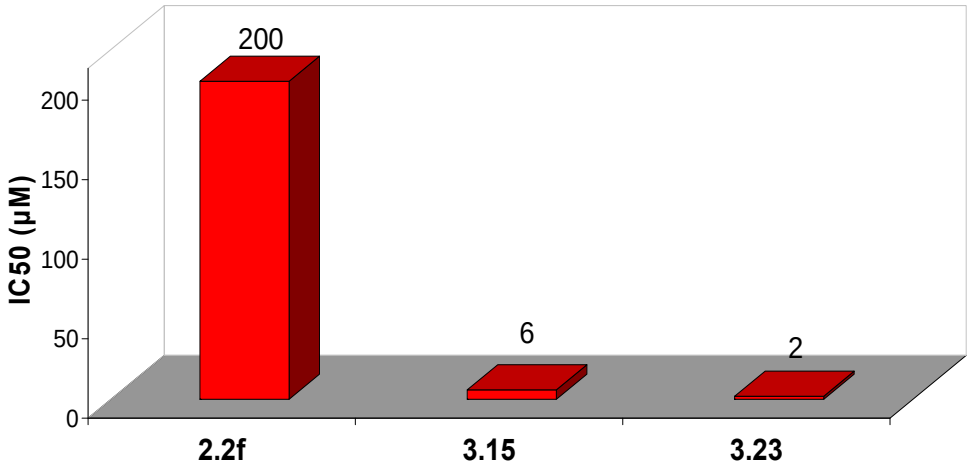
Comparing the monovalent **1.7b** with its multivalent forms significant improvement of the IC50 values are observed. The tetravalent presentation **3.16** with IC50 = 139 µM is more active by a factor of 6.5 in comparison with the monovalent ligand. The two hexavalent ligands **3.17** and **3.21** showed approximately the same potency with IC50 = 37 µM and 33 µM, which suggests, that unlike the valency, the shape of the multivalent molecule has a minor influence on the activity if this SPR technique is used for determination. The most remarkable improvement was observed in the case of the nonavalent system **3.20**, where the activity is higher by a factor of approximately 60. Besides the multivalent ligands **3.16**, **3.17, 3.20** and **3.21** which are based on

the triazole containing scaffolds, also the tetravalent compound **1.21a** (see first chapter) with the polyester backbone was tested. Its activity is similar to that of the new tetravalent construct **3.16**. It was previously found that the polyester scaffold of compound **1.21a** undergoes 30% hydrolysis after 3 hour at pH = 7.5.[51] The SPR measurements are done in pH = 8 what may cause partial decomposition of the polyester backbone, therefore the sample of **1.21a** was prepared immediately before the experiment to minimize the degradation.

Positive affinity improvement was also observed for multivalent forms of the bisamide derivative **2.2f**. The tetravalent and hexavalent constructs **3.15** and **3.23**, both based on the triazol-containing scaffolds were tested in the SPR competition assays.



2.2f

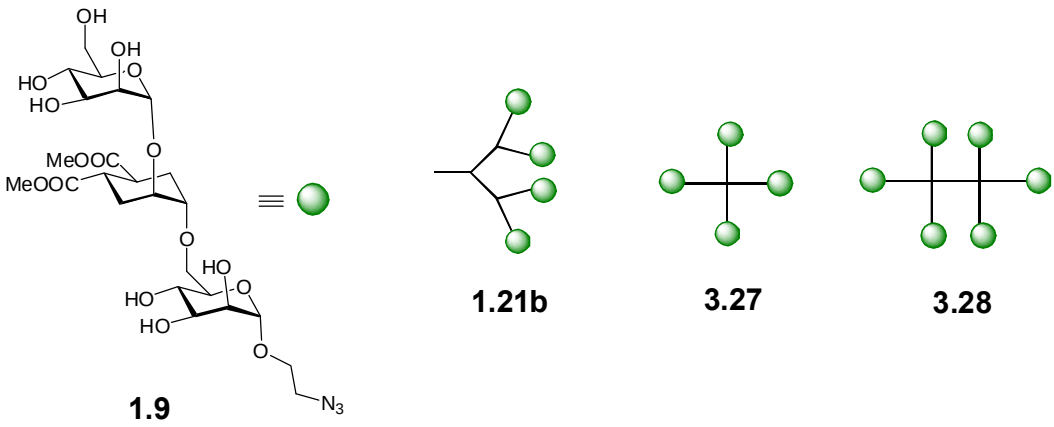


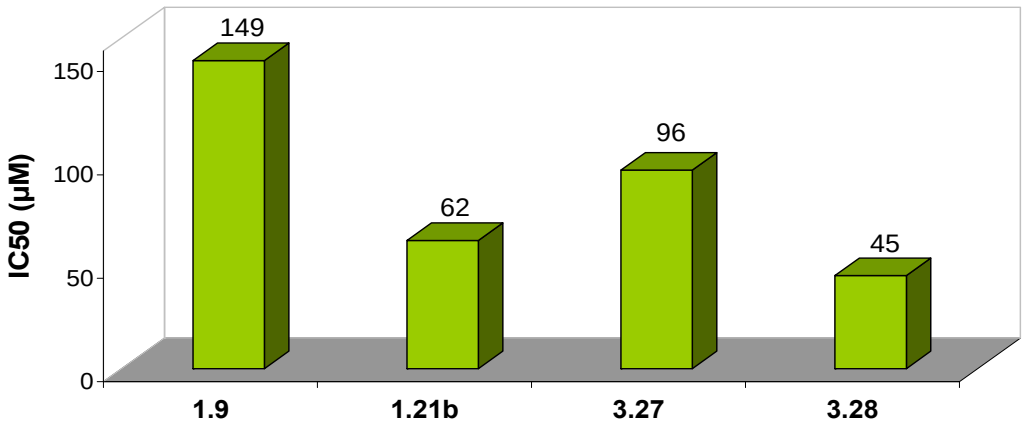
Graph 3.1 IC50 values of **2.2f** and glycodendrimers **3.15** and **3.23** measured by SPR

The affinity improvement was higher in comparison with **1.7b**. The tetravalent presentation **3.15** showed improvement by a factor of 33 with an IC50 = 6 µM. The hexavelent presentation **3.23** was found to be the most active DC-SIGN ligand from this series of multivalent compounds with an IC50 = 2 µM (Graph 3.1).

An interesting tendency was observed in the case of pseudotrisaccharide **1.9**. Although **1.9** is the most potent monovalent mannose mimic among those tested by SPR, its multivalent forms showed no or minimum improvement (Graph 3.2). The Boltorn-based tetravalent dendron **1.21b**

has an IC50 of 62 μM, over half the value of 149 μM observed for the monovalent ligand **1.9** in the same campaign. The tetravalent triazol-based construct **3.27** is close to activity to its monovalent counterpart (IC50 = 96 μM) and even the hexavalent dendron **3.28** is more active only by a factor of 3.





Graph 3.2 IC50 values of **1.9** and glycodendrimers **1.21b**, **3.27** and **3.28** measured by SPR

The puzzling behavior of multivalent constructs of the pseudotrisaccharide **1.9** was initially rationalized by the idea that this monovalent ligand is actually itself a divalent presentation of mannose units. If both end of the molecule can interact with the same DC-SIGN Ca^{2+} site, statiscal rebind can occur and this can explain the gain in affinity over the pseudodisaccharide **1.7** (Scheme 3.13A). Furthermore, when the reducing end terminus is connected to a multivalent scaffold, steric hindrance may prevent its interaction with the metal, and thus explain the lack of multivalency effect (3.13B). This interpretation, however, was not supported by the X-ray structure of the DC-SIGN:**1.9** complex, which showed a single binding mode, involving only the non-reducing end of the molecule, and fully superimposable with the structure of the DC:SIGN:**1.7** complex.[52] NMR data also showed that the X-ray structure explains at least 80%

of the STD interaction observed. A second binding mode may be present in solution, but it does not account for more than 20% of the data.[53] This distribution cannot account for the one order of magnitude increase in affinity observed when going from **1.7** to **1.9**. In an effort to understand the thermodynamics of the interaction, isothermal calorimetry (ITC) studies were performed in Grenoble, by Ieva Sutkeviciute. These titrations provided the surprising result that a 1:2 complex is formed in solution at the concentration used (Scheme 3.13C). Further exams, using analytical ultracentrifugation (AUC) of DC-SIGN tetramer in the presence of **1.9** confirmed the formation of a higher molecular weight species, consistent with a dimer of tetramers. These results led to the current working hypothesis: **1.9** apparently can bridge two DC-SIGN ECD using the non reducing end mannose for one ECD and the reducing end one for a second ECD (Figure 3.13C). Modeling studies did not find significant sterical clashes which would prevent the DC-SIGN ECD aggregation induced by **1.9**. This hypothesis would explain the low potency of multivalent ligands functionalized with **1.9**, since the reducing end of **1.9** is not available anymore for interaction (Scheme 3.13B).

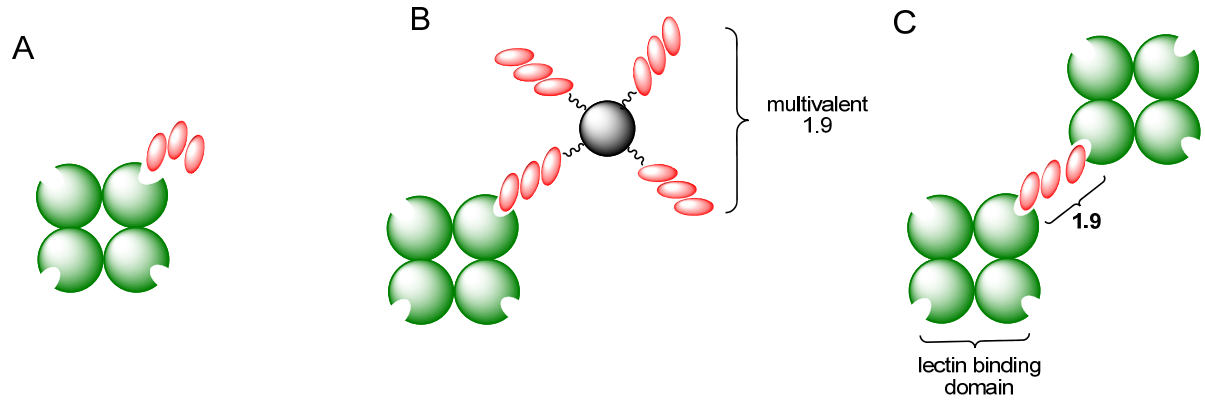
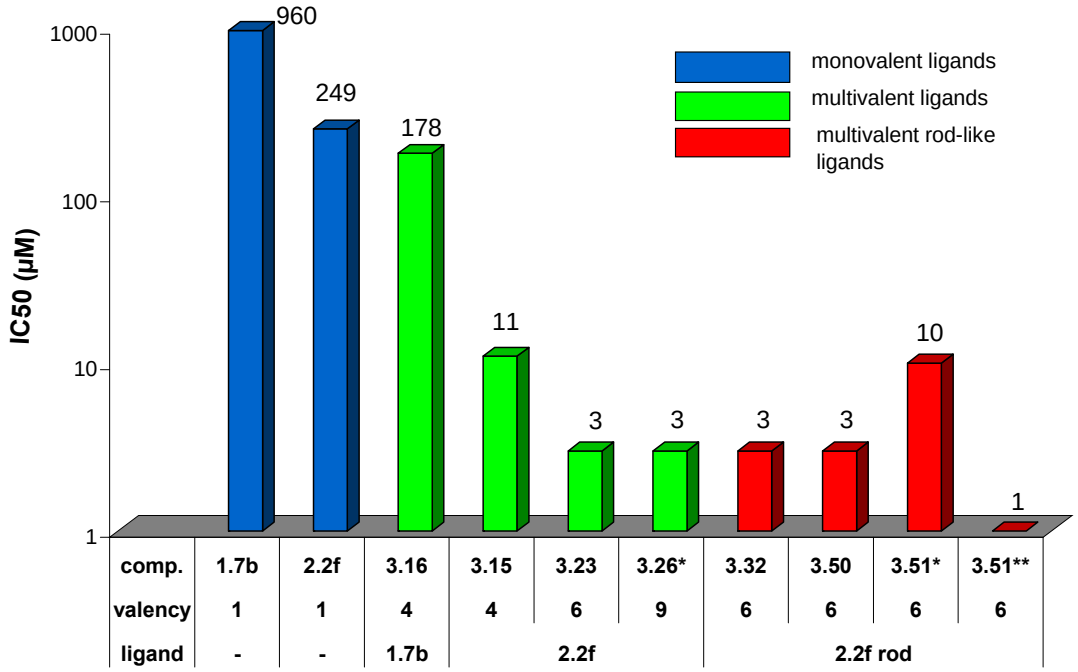


Figure 3.13 Schematic representation of A) binding of **1.9** to DC-SIGN B) binding of multivalent **1.9** to DC-SIGN C) aggregation of two DC-SIGN CRDs induced by monovalent **1.9**;

After the first SPR campaign the investigation focused on the preparation of multivalent forms of **2.2f** and on the use of rod-like structures as multivalent scaffolds. Once they were prepared, similarly to the majority of final compounds prepared during this thesis, the last series of multivalent DC-SIGN ligands **3.26**, **3.32**, **3.50**, **3.51** were sent to Grenoble to perform SPR experiments and determine their activities (Graph 3.3). The experimental setup was the same as the setup used for both monovalent ligands (second chapter) and multivalent ligands described above. The absolute IC50 value of the tested compounds can vary from campaign to campaign depending on the SPR chips used, however, their relative inhibition potency should remain same (or at least in same range). In order to follow the relative improvements of the activities of the

new molecules, some of the previously tested mono and multivalent DC-SIGN ligands were also examined along the rod-like structures.



Graph 3.3 IC50 values of monovalent ligands **1.7b**, **2.2f** and glycodendrimers **3.15**, **3.16**, **3.23**, **3.26**, **3.32**, **3.50** and **3.51** measured by SPR. * ligand not fully soluble in the buffer used. ** 20% DMSO added to the buffer.

The IC50 values found for the already tested monovalent (**1.7b**, **2.2f**) and multivalent (**3.15**, **3.16**, **3.23**) ligands are in good accordance with the SPR data coming from previous experiments. Structure **3.26** with nine copies of **2.2f** was tested for the first time in this campaign, however no improvement was found in comparison with the hexavalent **3.23** (IC50 = 3 μM) what can be attributed to the low solubility of **3.26**. The two hexavalent molecules **3.32** and **3.50** built on the shortest rod-like unit had the same activity as **3.23** (IC50 = 3 μM). This is in agreement with the data from molecular modeling that predicted that **3.32** and **3.50** like **3.23** are not long enough to reach two binding sites within one DC-SIGN CRD. Furthermore, the data show that the short PEG chain on the central aromatic ring in **3.50** has no effect on the activity. For the most complex molecule **3.51** significant solubility issues were reported. Using the same conditions as for the other compounds the IC50 was found to be 10 μM but, as with **3.26**, this number is likely to be incorrect. However adding 20% of DMSO to the buffer the compound was completely dissolved and the subsequent measurement showed IC50 = 1 μM.

3.4.2 Relative potency of multivalent ligands (β factor)

The relative potency, often called β factor, of multivalent ligands is an important value and can be considered as a measure of the multivalency effect.[5] It can be calculated as the activity of monovalent ligand divided by the activity of the corresponding multivalent ligand multiplied by the valency (equation 3.1).

$$\beta = \frac{IC50_{monov.comp}}{IC50_{multiv.comp.} \cdot valency}$$

Equation 3.1 Calculation of the β factor of multivalent molecules

The higher the β factor, the higher is the multivalency effect. The calculated β factor values of each tested multivalent compound are summarized in graph 3.4.

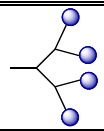
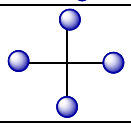
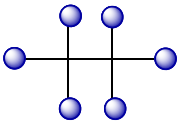
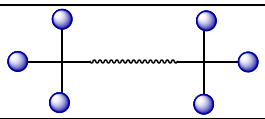
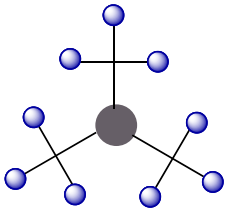
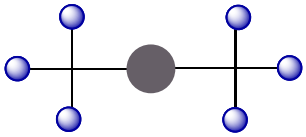
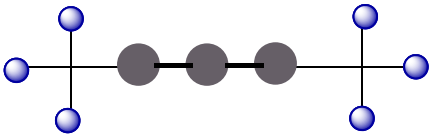
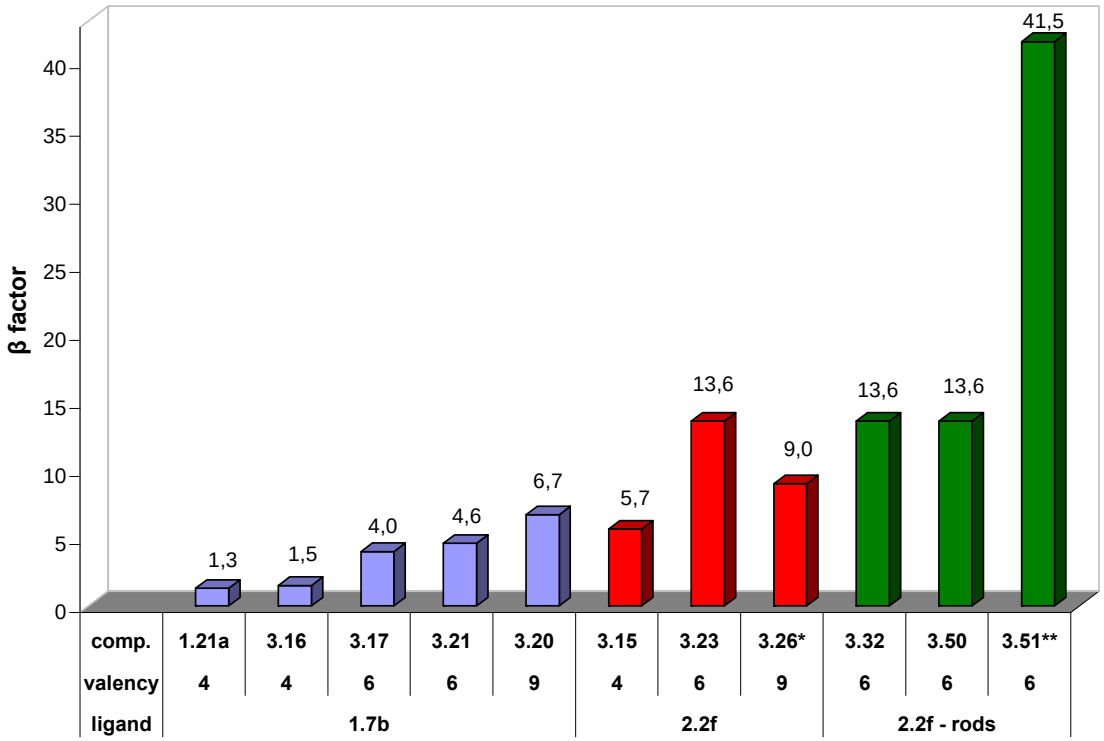
Scaffold	Monovalent ligand	Valency	Compound
	**1.7b**	4	**1.21a**
	**1.7b**	4	**3.16**
	2.2f	4	**3.15**
	**1.7b**	6	**3.17**
	2.2f	6	**3.23**
	**2.2f**	6	**3.21**
	**1.7b**	9	**3.20**
	2.2f	9	**3.26**
	**2.2f**	6	**3.32** (OCH_3)
			3.50 ($-OC_2H_4OC_2H_4OH$)
	**2.2f**	6	**3.51**

Table 3.1 Legend for graph 3.4



Graph 3.4 Relative potency (β factor) of multivalent DC-SIGN ligands tested by SPR. * ligand not fully soluble in the buffer used. ** 20% DMSO added to the buffer.

With multivalent ligands decorated with **1.7b** (**1.21a, 3.16, 3.17, 3.20** and **3.21**) a general trend of increasing β factor with growing valency has been observed and the highest relative potency was found for the nonavalent **3.20** (β factor = 6.7). On the other hand, among the erirthrytol derived scaffolds decorated with **2.2f** (**3.15**, **3.23** and **3.26**) the hexavalent **3.23** has the most efficient multivalency effect with a β factor of 13.6. Multivalent structures functionalized with pseudotrisaccharide **1.9** (not shown in Graph 3.4) have β factors below 1 which indicates that the multivalency does not improve the potency, on the contrary it has a significant negative effect. For the rod-like structures, the shorter ones **3.32** and **3.50** with six copies of **2.2f** have the same β factor as **3.23**, which is also hexavalent. However the hexavalent elongated rod-like dendrimer **3.51** has a remarkable relative potency of 41.5 which may be a good indication that this molecule can achieve simultaneous binding of two binding site.

In conclusion regarding the SPR data, multivalent structures bearing the previously optimized bis-amide **2.2f**, were found to be the most promising among the tested molecules. The relatively easy access and significant multivalency effect of the hexavalent **3.23** suggests that further biological or structural studies should focus on this molecule. On the other hand, multivalent

compounds with **1.7b** gave us an important information about the influence of the scaffold on the overall activity of the molecule. Ligand **1.7b** is relatively cheap in terms of synthesis, therefore it can be used for further screening of novel multivalent scaffolds. In the case of **1.9**, it was found that going multivalent does not bring any additional improvement in the potency, therefore any further investigation of more complex structures with **1.9** should be abandoned.

Regarding the rod-like multivalent structures it is useful to note that the kind of SPR experiments performed so far, for the reason that are fully described below, are not completelly appropriate for the determination of improvements which are coming from the simultaneous inhibition of two binding sites in one DC-SIGN tetramer. Nevertheless, the results from the last SPR campaigns are rather interesting and several suggestions can be proposed for further investigation. One of them is related with the bad solubility of some of the multivalent compounds bearing bis amide **2.2f**. To avoid this problem the rod-like structures should be decorated with **1.7b** which shows good water solubility in all of its multivalent presentations. The lower potency of **1.7b** in comparison with **2.2f** could result in higher IC50 values, however the goal in this case is to achieve the "prove of concept". The second suggestion is more complex and is related with the SPR method used for the activity determination of ligands. The potency of our ligands were measured by SPR experiments in which both the DC-SIGN ECD and its potential inhibitor are flowing in the buffer media whereas the competitive inhibitor (1-α-trimannoside) is immobilized on the surface of the chip (for more details see chapter 2). This suggests that the increase of activity in the case of multivalent compounds can be a result of both proximity effect and aggregation of two or more DC-SIGN ECDs promoted by the multivalent ligand (Figure 3.14A).

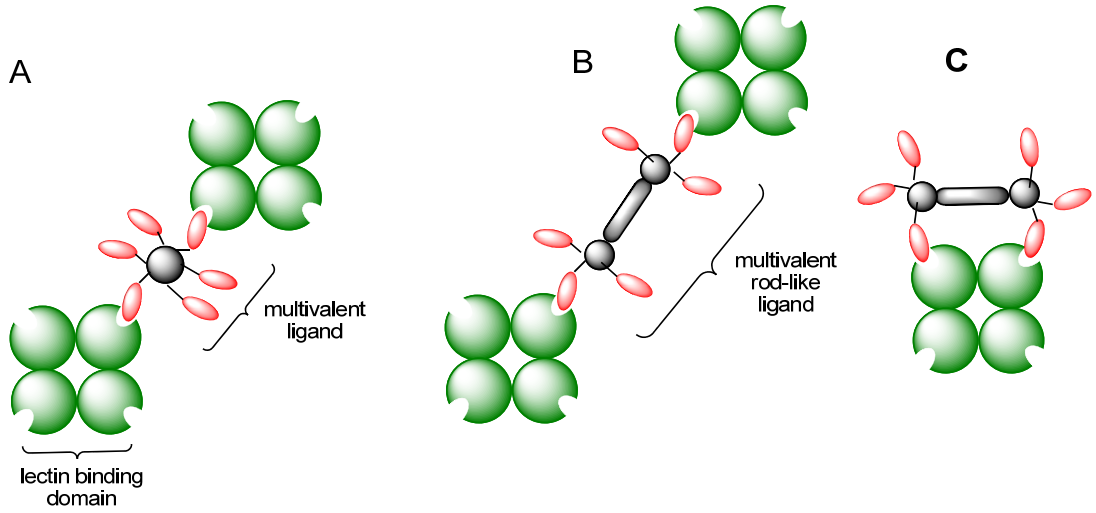


Figure 3.14 Schematic representation of A) DC-SIGN ECD aggregation induced by a multivalent ligand B) DC-SIGN ECD aggregation induced by a multivalent rod-like ligand C) simultaneous inhibition of two binding sites in one DC-SIGN ECD by a multivalent rod-like ligand

Based on this hypothesis, multivalent compounds containing rigid spacers can inhibit either two binding sites of two DC-SIGN tetramers (Figure 3.14B, similarly to 3.14A) or two binding sites of the same DC-SIGN tetramer (Figure 3.14C). It is difficult to predict which of these hypothetical binding forms has a bigger effect on the outcoming result, but most probably the impact is similar. Therefore it is difficult to recognize those compounds which are able to bind two binding sites within one CRD using this experimental setup. For this purpose the setup should be changed in a way where the DC-SIGN ECD is immobilized on the chip and the potential ligand is dissolved in the media. The DC-SIGN ECD immobilization leads to an oriented surface in which the density of the DC-SIGN can be controlled.[54,55]

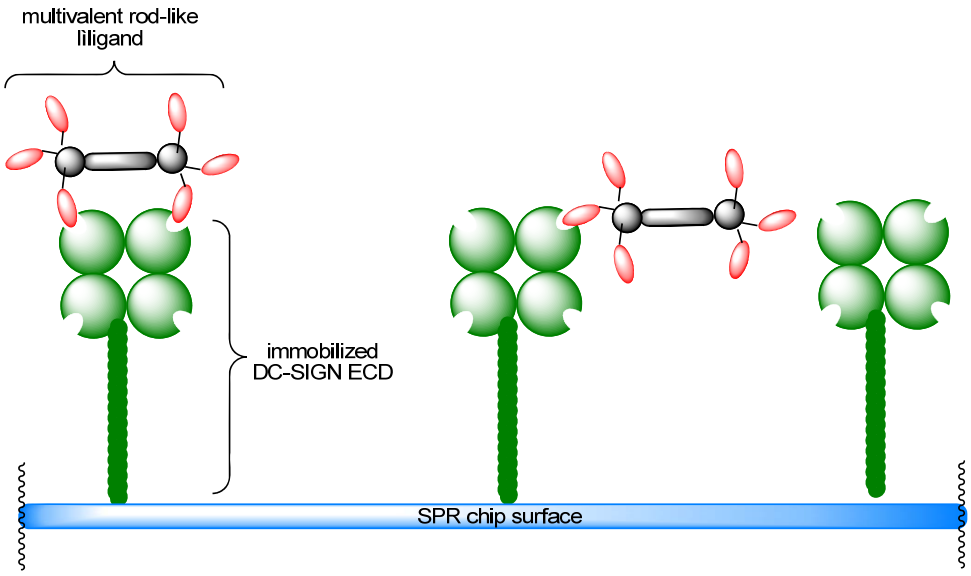


Figure 3.15 Schematic representation of an oriented surface immobilized with DC-SIGN which are inhibited by a multivalent rod-like structure

Having the appropriately low density of immobilized DC-SIGN ECD, multivalent compounds should be able to bind two binding sites only within one CRD. This should result in a much lower IC50 for those compounds which can perform the simultaneous binding.

3.4.3 Cell studies

Some of the synthesized compounds were tested for the ability to inhibit HIV transmission in an in vitro trans infection assay. Tests were performed by Angela Berzi in the lab of Prof. Clerici at the university of Milano.

B-THP-1/DC-SIGN cells are derived from B-THP-1 human B cell line by transfection of DC-SIGN expression vector in order to express high levels of the DC-SIGN receptor at the surface of these cells. This cell line supports efficient DC-SIGN mediated HIV transmission and it is a widely-used model system to mimic HIV capture and transmission to T-lymphocytes by dendritic cells.[56] In a first series of experiments, B-THP-1/DC-SIGN or BTHP1 cells pre-incubated for 30 minutes in the presence or in the absence of the DC-SIGN inhibitors were subsequently exposed to HIV (the R5 tropic laboratory-adapted strain HIV-1 BaL) in the continued presence of inhibitors. Mannan is known to inhibit DC-SIGN mediated viral infection[57,58,59] and was used as positive control. Non transfected B-THP-1 cells were used as a negative control and, as expected, did not transmit infection.

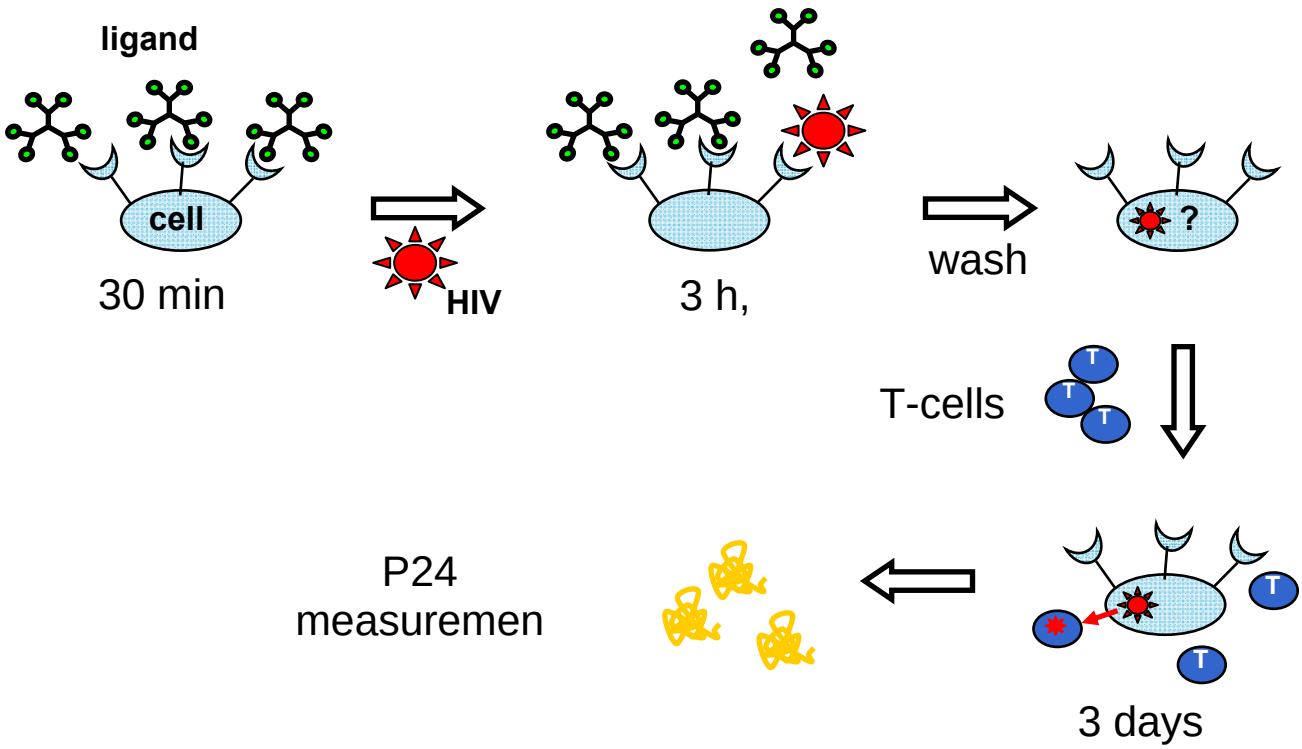
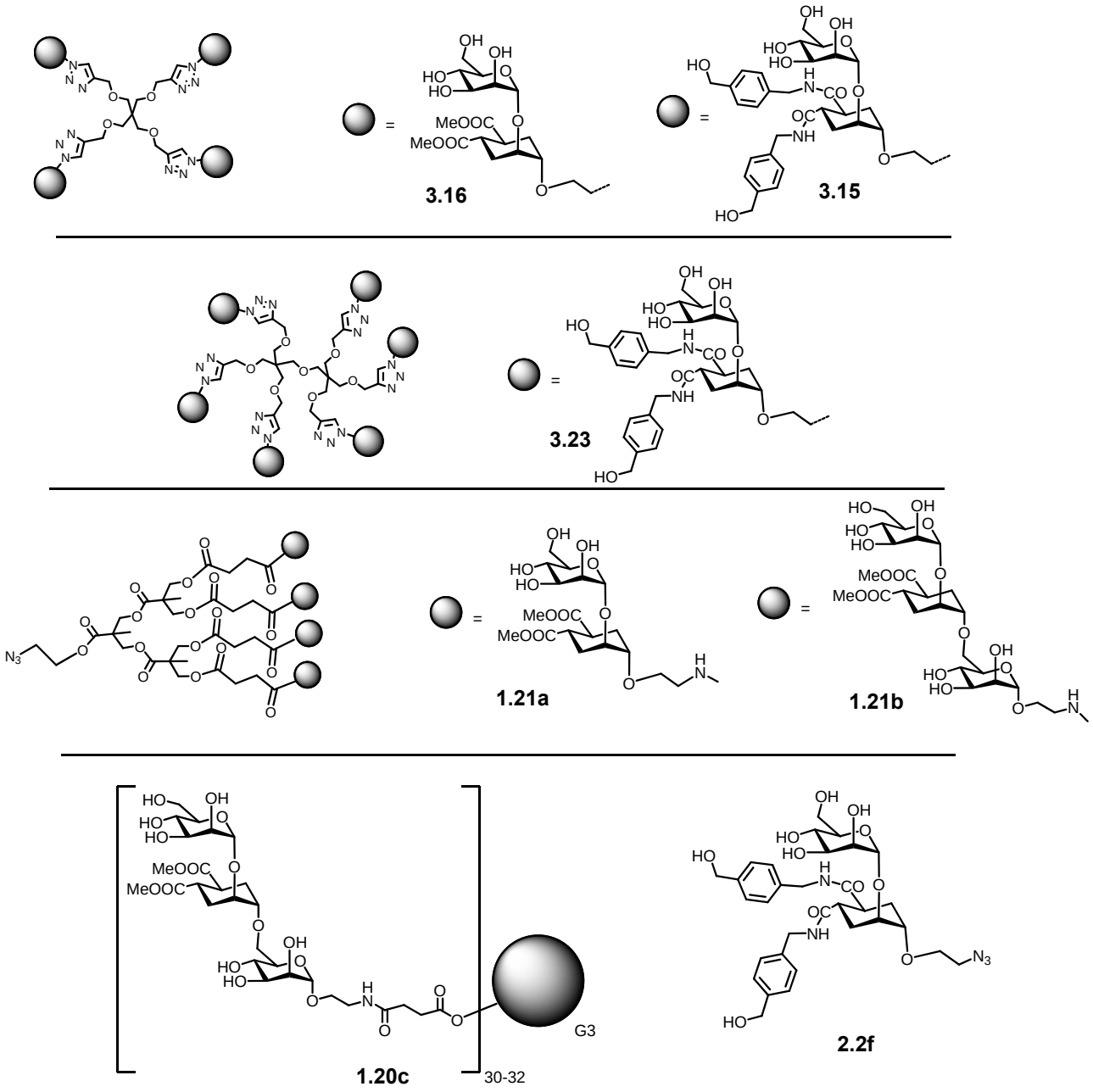
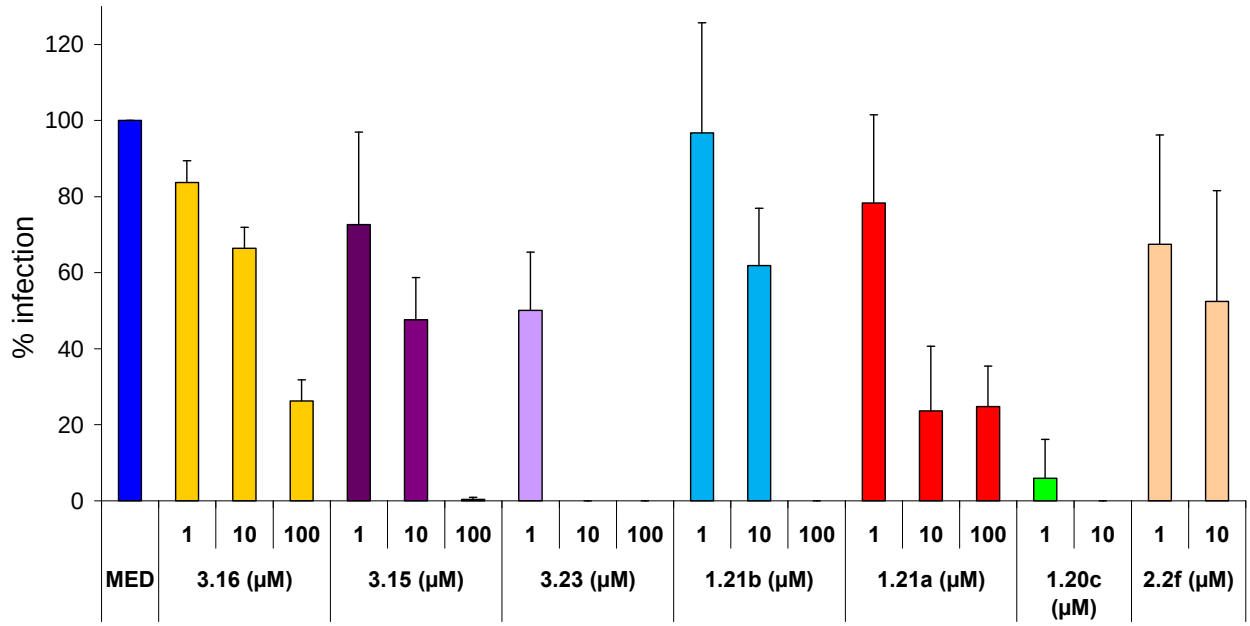


Figure 3.16 Schematic representation of the experimental setup of the HIV trans infection studies

After washing, the B-THP-1/DC-SIGN cells were co-cultured with activated CD4+ T lymphocytes from healthy volunteer donors. Viral infection of CD4+ T lymphocytes was assessed by measuring the concentration of the HIV core protein p24 in the co-culture supernatants by ELISA. **p24**, immunologically distinct from the protein of most other retrovirus, is a major structural core component of HIV-1 and is estimated to be present at 2000-4000 molecules in each virion. The measurement of p24 levels is therefore a commonly exploited method to verify the successful infection by the virus. Each point was obtained in triplicate using CD4+ T lymphocytes from three different healthy donors, and each compound was tested at different concentration (1 μM, 10 μM and 100 μM,). The HIV trans infection studies focused on those multivalent structures which were found to be the most active in the SPR experiment, namely compounds **3.15** and **3.23**. Besides the structures containing bis-amide **2.2f** and the selected standard **3.16**, several other molecules which had been previously tested were included into the campaign (Scheme 3.35).



Scheme 3.35 Glycodendrons and glycodendrimers tested by HIV trans infection studies



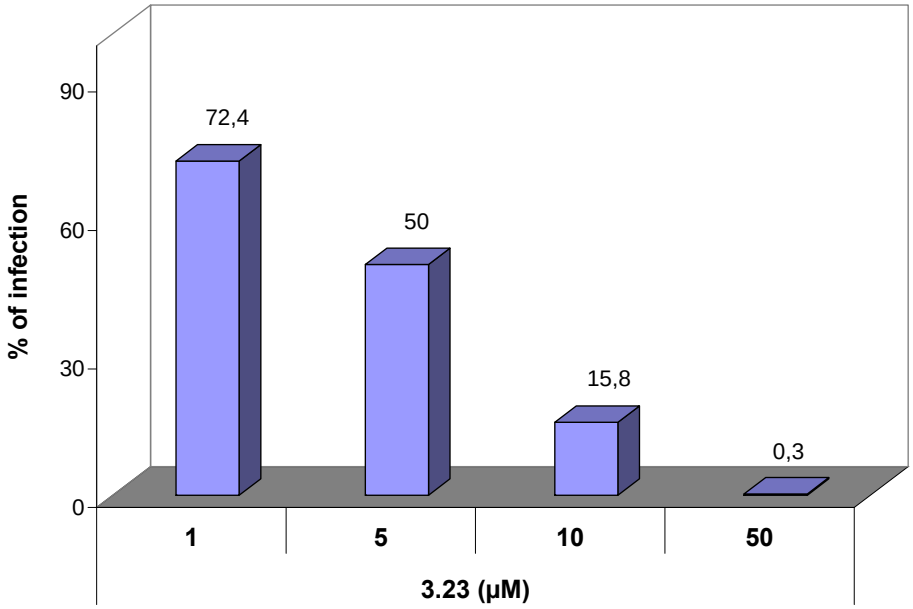
Graph 3.5 Level of HIV infection after the treatment with mono and multivalent DC-SIGN ligands in trans HIV infection studies

Tetravalent **3.16** reduced the infection to 66% and 26% at 10 μM and 1 μM concentration, respectively. After treatment with compound **3.15,** bearing four copies of **2.2f**, 47 % of infection was determined at 10 μM and almost no infection took place at 100 μM concentration. The most impressive inhibition of the trans HIV infection was observed in the case of **3.23** (hexavalent **2.2f**). At 1 μM concentration the infection was reduced to 50 %, and at 10 μM and 100 μM the infection was completely suppressed. For comparison, previously prepared and reported multivalent ligands **1.21a** and **1.21b** were also tested. Pseudotrisaccharide **1.9** in its tetravalent form, **1.21b**, showed similar activity to **3.16** at 1 μM and 10 μM but at 100 μM no infection was observed. For pseudodisacharide derivative **1.21a**, connected to the same polyester based scaffold as **1.21b**, very similar activity to compound **3.16** at 1 μM and 100 μM was found, but at 10 μM higher activity was observed. The most complex multivalent ligand **1.20c** with approximately 31 copies of **1.9** showed very strong inhibition potency. No infection was observed at 10 μM concentration and only 5.9 % of infection took place at 1 μM what is in a good agreement with previously reported data. [60]

Activities obtained in the trans infection studies for **3.16**, **3.15** and **3.23** are in relatively good correlation with the data from the SPR measurements. Approximately 50% of inhibition was determined for the tetravalent presentation of **2.2f** at 10 μM, leading to the conclusion that the IC50 of **3.15** is 10 μM. For the hexavalent presentation **3.23** the IC50 can be set to 1 μM. The most potent inhibitor is still the Boltorn type dendrimer **1.20c**, however this compound is bearing

31 copies of structurally complex **1.9** and moreover, its synthesis is much more demanding in comparison with the multivalent structures based on click chemistry. On the other hand, compound **3.23**, with only 6 copies of **2.2f,** approaches the activity of **1.20c** what makes **3.23** the most efficient multivalent ligand.

DC-SIGN plays an important role also during the transmission of dengue virus.[61] This disease is primarily transmitted by mosquitoes and the symptoms are fever, muscle and joint pains and skin rash. In order to examine the potential activity of our compounds to inhibit dengue virus infection, compound **3.23** was sent to the group of Dr. Ali Amara[62] (INSERM, Paris) to perform some initial studies. The experiments were carried out by a PhD, student Rasika Mohan Ramdasi. Raji DC-SIGN cells were infected with dengue virus serotype-2 in the presence of **3.23** at different concentrations (Graph 3.6).



Graph 3.6 Level of dengue virus infection after the treatment with **3.23** at different concentration

Ligand **3.23** showed concentration dependent antiviral activities. At 50 µM concentration the infection was almost completely blocked and the IC50 was found to be 5 µM proving that multivalent ligands functionalized with **2.2f** can inhibit both HIV and dengue virus at low micromolar range.

The citotoxicity of **3.23** was evaluated by cell labeling after the incubation period with a specific marker for death cells, 7-aminoactinomicin D (7-AAD). Percentage of 7-AAD positive cells (apoptotic cells) did not change significantly in the absence of the compound or in its presence up to 50 µM.

The infection studies confirmed that **3.23** is the most interesting ligand. Both the multivalent scaffold **3.3** and monovalent ligand **2.2f** are synthetically achievable in gram scale in our laboratory and only one functionalization is needed to obtain DC-SIGN ligand **3.23**. Moreover, the final structure **3.23** is chemically stabile unlike the multivalent compounds based on the polyester backbone.

3.5 Experimental part

3.5.1 General

Dichloromethane, methanol, N,N-diisopropylethylamine and triethylamine were dried over calcium hydride; THF was distilled over sodium, N,N-dimethylacetamide (DMA) was dried over activated molecular sieves (4Å). Reactions requiring anhydrous conditions were performed under nitrogen. ^{1}H and ^{13}C spectra were recorded at 400MHz on a Bruker AVANCE-400 and 300MHz on Brucker DPX-300 instrument. Chemical shifts (δ) for ^{1}H and ^{13}C spectra are expressed in ppm relative to internal standard ($CDCl_3$: 7.24 for ^{1}H and 77.23 for ^{13}C; CD_3OD: 3.31 for ^{1}H and 49.15 for ^{13}C; D_2O: 4.80 for ^{1}H). Signals were abbreviated as s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Mass spectra were obtained with a ThermoFisherLCQapparatus (ESI ionization), or iontrap ESI Esquire 6000 from Bruker, or a Microflex apparatus (MALDI ionization) from Bruker, or Apex II ICR FTMS (ESI ionization-HRMS). Specific optical rotation values were measured using a Perkin-Elmer 241, at 589 nm, in a 1 dm cell. Thin layer chromatography (TLC) was carried out with pre-coated Merck F254 silica gel plates. Flash chromatography (FC) was carried out with Macherey-Nagel silica gel 60 (230–400 mesh).

Numbering: The numbering used in the NMR characterizations is indicated in the structures showed after the procedures. Sugar signals were numbered as customary; cyclohexane protons are indicated with the letter D followed by numbers. The unusual numbering of the pseudo-saccharide derivatives in the NMR characterizations was adopted to facilitate comparison with the native disaccharide. In the case of rod-like dendrimers **3.32**, **3.49**, **3.50** and **3.51** the aryl-ethynyl units are indicated with the letter R followed by numbers, and the polyethyleneglycol chains with letter the G followed with numbers. In the names of the compounds the conventional numbering is used.

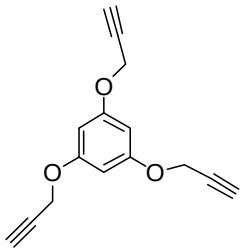
Click reaction: In the optimized procedure of the copper(I) catalyzed 1,3-dipolar cycloadition the starting materials and reagents were added to the reaction mixtures as solutions in water or THF. Monovalent lignds (**1.7b**, **1.9** and **2.2f**) and dendrons (**3**.**19**, **3.25**) with azide groups were added as solids. The reagents were added to the reaction in the following order: multivalent scaffold (THF), TBTA (THF), copper(II) sulphate (water), sodium ascorbate (water) and finally the azide derivative. The water was degassed by bubbling with nitrogen and the THF was freshly distilled. The reactions were stirred under nitrogen atmosphere and protected form light. After reaction completion the mixtures were loaded directly on SEPHADEX LH-20 columns (55 cm x

3.5cm) to purify the products by size exclusion chromatography. In order to remove copper residues from the products reverse phase chromatography was performed (C18, eluent: H_2O with gradient of methanol or acetonitrile) or to the solution of product in methanol a metal scavenger[42] (such as Quadrasil™ MP) was added and stirred for 5 min. The scavenger was filtered off through a cotton pad and the filtrate was concentrated to obtain the product. Compounds **3.2**, **3.4**, **3.16 - 3.21** and **3.28** were prepared during my secondment in the group of Dr. Javier Rojo (Seville)[18], whereas the rest of the molecules were synthesized in the group of professor Anna Bernardi (Milano). Scaffolds **3.1** and **3.3** were prepared by Renato Ribeiro (group of Dr. Javier Rojo) in Seville.

3.5.2 Synthesis of multivalent scaffolds 3.1-3.5

3.5.2.1 1,3,5-Tris(2-propynyloxy)benzene, 3.1[19]

To a solution of 1,3,5-trihydroxybenzene (0.265 g, 1.58 mmol, 1 eq.) in dry DMF (4 mL) potassium carbonate (anhydrous, 0.628 g, 6.35 mmol, 4.2 eq.) was added at room temperature. The solution was stirred at 50ºC for 1 h then propargyl bromide (0.680 mL, 6.35 mmol, 4.2 eq.) was slowly added. The mixture was stirred at 65ºC for 16 h. The reaction was quenched by slow addition of distilled water then extracted with diethyl ether (3 x 30 mL). The combined organic phases were dried over sodium sulphate and concentrated under reduced pressure. The crude was purified by flash chromatography (silica, hex:EA = 9:1) to afford 60 mg of pure product.



3.1

Yield: 25 %

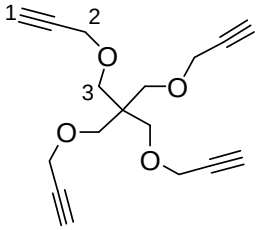
MS (ESI) calculated for $[C_{15}H_{12}O_3Na]^+$: 263.2; found = 262.9.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.27 (s, 3H, Ar-H), 4.67 (d, 6H, OCH_2, $J_{2\text{-}1}$ = 2.4 Hz), 2.53 (t, 3H, CCH, $J_{2\text{-}1}$ = 2.4 Hz)

3.5.2.2 Tetrakis(2-propynyloxymethyl)methane, 3.2[20]

To a solution of pentaeritritol (0.1 g, 0.74 mmol, 1 eq.) in dry DMF (20 mL) sodium hydride (0.176 g, 3.99 mmol, 5.4 eq.) was added under argon at -5ºC. The solution was stirred at -5ºC for 1 h then propargyl bromide (0.42 mL, 4.41 mmol, 6 eq.) was added. The reaction mixture kept at

-5ºC for 20 min then, was let to warm up to room temperature and stirred for 19 h. The reaction was quenched by slow addition of water then extracted with diethyl ether (3 x 30 mL). The combined organic phases were dried over sodium sulphate and concentrated under reduced pressure. The crude was purified by flash chromatography (slica, hex:EA = 8.5:1.5) to afford 130 mg of pure product.



3.2

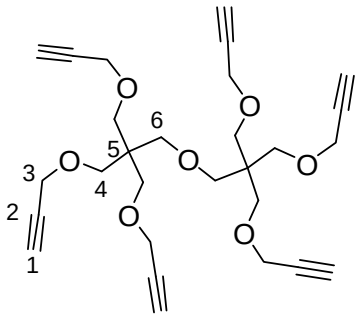
Yield: 66 %

MS (ESI) calculated for $[C_{17}H_{20}O_4Na]^+$: 311.1; found = 311.1.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.12 (d, 8H, H_2, $J_{2\text{-}1}$ = 2.4 Hz), 3.53 (s, 8H, H_3), 2.40 (t, 4H, H_1, $J_{2\text{-}1}$ = 2.4 Hz)

3.5.2.3 Hexa(2-propynyloxymethyl) bispentaeritritol, 3.3

To a solution of bispentaeritritol (0.3 g, 1.18 mmol, 1 eq.) in dry DMF (20 mL) sodium hydride (0.34 g, 14 mmol, 11.8 eq.) was added under argon at -5ºC. The solution was stirred at -5ºC for 1 h then propargyl bromide (1.15 mL, 14 mmol, 11.8 eq.) was added and the mixture was kept at -5ºC for additional 20 min. The reaction was let to warm up to room temperature and stirred for 19 h. The reaction was cooled to 0ºC, quenched by slow addition of distilled water and extracted with diethyl ether (3 x 30 mL). The combined organic phases were dried over sodium sulphate and concentrated under reduced pressure. The crude was purified by flash chromatography (slica, hex:EA = 9:1 and 8.5:1.5) to afford 130 mg of pure product.



3.3

Yield: 65 %

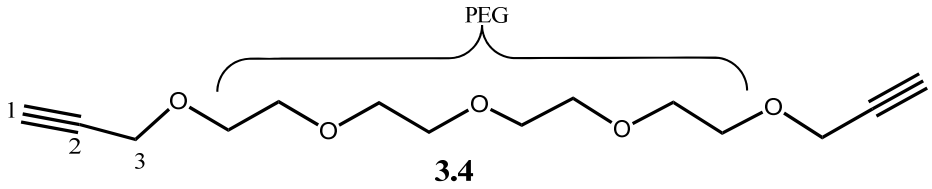
MS (HRMS) calculated for $[C_{28}H_{34}O_7Na]^+$: 483.2383; found = 483.2379.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.12 (d, 12H, H_3, $J_{3\text{-}1}$ = 2.4 Hz), 3.52 (s, 12H, H_4), 3.38 (s, 4H, H_6), 2.53 (t, 6H, H_1, $J_{3\text{-}1}$ = 2.4 Hz).

^{13}C NMR (75 MHz, $CDCl_3$): δ = δ 80.2 (C_1); 74.3 (C_2); 69.9 (C_3); 69.3 (C_4); 58.9 (C_6); 45.2 (C_5).

3.5.2.4 4,7,10,13,16-Pentaoxanonadeca-1,18-diyne, 3.4[22]

To a solution of ditosylate **3.14** (60 mg, 0.119 mmol, 1 eq.) in propargyl alcohol (0.5 mL) potassium carbonate (66 mg,, 0.477 mmol, 4 eq.) was added. The solution was stirred at 45ºC overnight. The reaction was diluted with ethyl acetate washed with water and brine. The organic phases was dried over sodium sulphate and concentrated under reduced pressure. The crude was purified by flash chromatography (silica, hexane with gradient of ethyl acetate from 20% to 50%) to afford 30 mg of pure product.



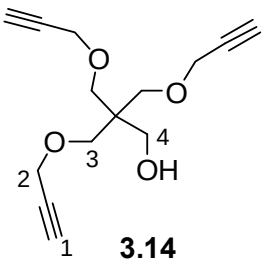
3.4

Yield: 93 %

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.13 (d, 6H, H_3, $J_{3\text{-}1}$ = 2.4 Hz), 3.71 - 3.54 (m, 16H, H_{PEG}), 2.40 (t, 2H, H_1, $J_{3\text{-}1}$ = 2.4 Hz).

3.5.2.5 Tris(2-propynyloxymethyl)hydroxymethyl methane, 3.14[21]

To a solution of pentaeritritol (0.1 g, 0.74 mmol, 1 eq.) in dry DMF (20 mL) sodium hydride (0.092 g, 2.29 mmol, 3.1 eq.) was slowly added under argon at 0ºC. The solution was stirred at 0ºC for 1 h then propargyl bromide (0.25 mL, 2.29 mmol, 3.1 eq.) was added. The reaction mixture was stirred at 0ºC for 20 min then, was let to warm up to room temperature and stirred for 19 h. The reaction was quenched by slow addition of distilled water then extracted with diethyl ether (3 x 30 mL). The combined organic phases were dried over sodium sulphate and concentrated under reduced pressure. The crude was purified by flash chromatography (silica, hex:EA = 8:2, 7:3) to afford 114 mg of pure product.



3.14

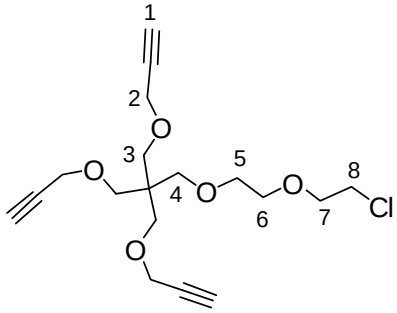
Yield: 62 %

MS (ESI) calculated for $[C_{14}H_{18}O_4Na]^+$: 273.3; found = 273.1.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.14 (d, 6H, H_2, $J_{3\text{-}1}$ = 2.3 Hz), 3.69 (s, 2H, H_4), 3.57 (s, 6H, H_3), 2.24 (t, 3H, H_1, $J_{3\text{-}1}$ = 2.3 Hz).

3.5.2.6 2-(2-Cloroethoxy)ethoxymethyl tris(2-propynyloxymethyl)methan, 3.5[21]

To a solution of **3.14** (50 mg, 0.2 mmol, 1 eq.) in bis(2-cloroethyl)eter (1 mL) $(nBu)_4N.HSO_4$ (136 mg, 0.4 mmol, 3.1 eq.) and NaOH (aq. 50%, 1 mL) was slowly added at room temperature. The reaction mixture was virgously stirred at 40ºC for 18 h then DCM (6 mL) and water (6 mL) were added. The organic phase was separated and washed with distilled water (2 x 10 mL), dried over sodium sulphate and concentrated under reduced pressure. The crude was purified by flash chromatography (silica, hex:EA = 9:1, 8.5:1.5) to afford 60 mg of pure product.



3.5

Yield: 80 %

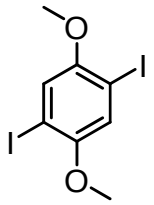
MS (ESI) calculated for $[C_{18}H_{25}ClO_5Na]^+$: 379.8; found = 379.2.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.05 (d, 6H, H_2, $J_{3\text{-}1}$ = 2.4 Hz), 3.70 (t, 2H, H_8, $J_{7\text{-}8}$ = 5.9 Hz), 3.61 – 3.50 (m, 6H, H_5, H_6, H_7), 3.46 (s, 6H, H_3), 3.40 (s, 2H, H_4), 2.33 (t, 3H, H_1, $J_{3\text{-}1}$ = 2.4 Hz).

3.5.3 Synthesis of rod-like scaffolds 3.7a-b, 3.41 and 3.42

3.5.3.1 1,4-diiodo-2,5-dimethoxybenzene, 3.30[43a]

To a solution of H_5IO_6 (10 g, 42 mmol, 0.6 eq.) in methanol (70 mL) iodine (11.5 g, 90 mmol, 1.25 eq.) was added. The resulting solution was stirred at room temperature for 10 min then 1,4-dimethoxybenzene (10 g, 72 mmol, 1 eq.) was added. The reaction was heated to reflux for 4 h then stirred at room temperature overnight. The mixture was poured into an aqueous solution of $Na_2S_2O_5$ (20 g in 200 mL of water) and the resulting precipitates were filtered off, washed with a small amount of water (20 mL) and methanol (10 mL) and recrystallized from isopropanol (300 mL) to afford 11 g of pure product.



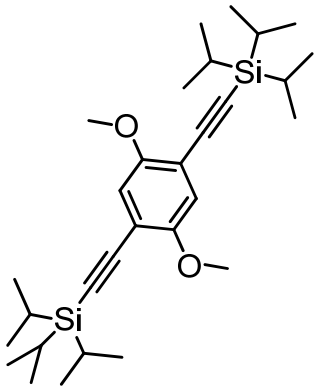
3.30

Yield: 40 %

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.18 (s, 2H, Ar-H), 3.81 (s, 6H, OCH_3).

3.5.3.2 1,4-dimethoxy-2,5-bis[2-[tris(1-methylethyl)silyl]ethynyl]benzene, 3.31[43a]

3.30 (200 mg, 0.51 mmol, 1 eq.), $Pd(PPh_3)_4$ (23 mg, 0.02 mmol, 0.04 eq.), CuI (4 mg, 0.02 mmol, 0.04 eq.), and PPh_3 (14 mg, 0.05 mmol, 0.1 eq.) were placed into the reaction flask and dried under vacuum. The reagents were dissolved by addition of toluene (3 mL) and finally ethynyltriisopropylsilane (TIPS-acetylene, 187 mg, 1.02 mmol, 2 eq.), and TEA (2 mL) were added under nitrogen. The reaction was heated to 120°C for 24 h. The solvent was removed under reduced pressure and the resulting crude was purified by flash chromatography (silica, hexane with gradient of ethyl acetate from 0 to 20%) to afford 200 mg of product.



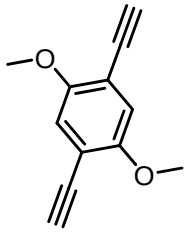
3.31

Yield: 83 %

^{1}H NMR (400 MHz, CDCl3): δ = 6.86 (s, 2H, Ar-H), 3.79 (s, 6H, OCH$_3$), 1.3 - 0.94 (m, 6H, Si-CH), 1.11 – 1.13 (m, 36H, CH(CH$_3$)$_2$).

3.5.3.3 1,4-Diethynyl-2,5-dimethoxybenzene, 3.7a [43a]

To a solution of **3.31** (100 mg, 0.2 mmol, 1 eq.), in THF (1 mL) a solution of TBAF in THF (1 M, 1 mL, 1 mmol, 5 eq.) was added. The resulting solution was stirred for 2 h at room temperature. The solvent was removed under reduced pressure and the crude was purified by flash chromatography (silica, hexane with gradient of ethyl acetate from 0 to 50%) to afford 35 mg of pure product.



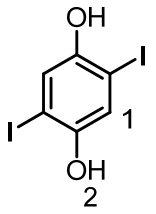
3.7a

Yield: 95 %

^{1}H NMR (400 MHz, CDCl3): δ = 6.89 (s, 2H, Ar-H), 3.84 (s, 6H, OCH$_3$), 3.38 (s, 2H, CCH).

3.5.3.4 1,4-Dihydroxy-2,5-diiodobenzene, 3.33 [63]

To a solution of **3.30** (7 g, 17.9 mmol, 1 eq.), in DCM (70 mL) a solution of BBr_3 in DCM (1 M, 71 mL, 71 mmol, 4 eq.), was added at -78°C under nitrogen. The reaction was let to warm up to room temperature and stirred overnight. The reaction was quenched by addition of water at 0°C then the mixture was diluted with ethyl acetate, washed with water and brine, dried over sodium sulphate and concentrated under reduced pressure to afford 5.6 g of pure product.



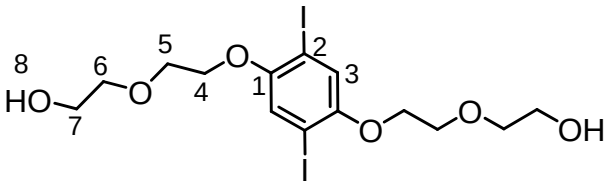
3.33

Yield: 86 %

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.27 (s, 2H, Ar-H), 4.89 (br s, 2H, OH).

3.5.3.5 1,4-bis[2-(2-hydroxyethoxy)ethoxy]-2,5-diidobenzene, 3.34[64]

A solution of **3.33** (1.8 g, 4.97 mmol, 1 eq.), 2(2-Chloroethoxy)ethanol (3.1 g, 24.86 mmol, 5 eq.) and K_2CO_3 (2.7 g, 19.88 mmol, 4 eq.) in DMF (10 mL) was stirred at 70°C overnight. The solvent was removed under reduced pressure, the crude residue was taken up with ethyl acetate and transferred to a separatory funnel, washed with aq. $Na_2S_2O_5$ (10%), water and dried over sodium sulphate. The solvent was removed under reduced pressure and the crude was purified by flash chromatography (silica, chloroform with gradient of methanol from 0% to 10%) to afford 1.5 g of pure product.



3.34

Yield: 57 %

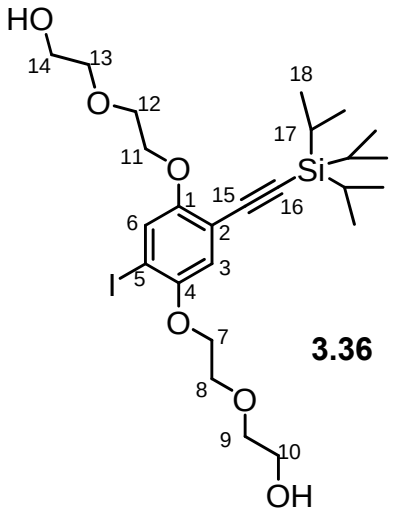
MS (ESI) calculated for $[C_{14}H_{20}I_2O_6Na]^+$: 561.1; found = 561.3

^{1}H NMR (400 MHz, $CDCl_3$):): δ = 7.22 (s, 2H, H_3), 4.11 – 4.07 (m, 4H, H_4), 3.88 – 3.84 (m, 4H, H_5), 3.76 – 3.72 (m, 4H, H_7), 3.71 – 3.76 (m, 4H, H_6), 1.67 (s, 2H, H_8).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 123.6 (C_3); 86.7 (C_2); 72.8 (C_6); 70.3 (C_4); 69.7 (C_5); 62.1 (C_7).

3.5.3.6 1,4-bis[2-(2-hydroxyethoxy)ethoxy]-2-[tris(1-methylethyl)silyl]ethynyl)-5-idobenzene, 3.36 and 1,4-bis[2-(2-hydroxyethoxy)ethoxy]-2,5-bis[tris(1-methylethyl)silyl]ethynyl)benzene, 3.35

3.34 (50 mg, 0.093 mmol, 1 eq.), $Pd(PPh_3)_4$ (4 mg, 0.003 mmol, 0.04 eq.), CuI (6.5 mg, 0.003 mmol, 0.04 eq.), and PPh_3 (2.3 mg, 0.009 mmol, 0.1 eq.) were placed into the reaction flask and dried under vacuum, then the reagents were dissolved in toluene (0.6 mL) and finally ethynyltriisopropylsilane (TIPS-acetylene, 25.4 mg, 0.139 mmol, 1.5 eq.), and TEA (0.2 mL) were added under nitrogen. The reaction was heated to 50°C for 5h. The reaction was diluted with EA, filtered through a silica pad and the filtrate was concentrated under reduced pressure. The resulting crude was purified by flash chromatography (silica, hexane with gradient of ethyl acetate from 0 to 90%) to afford 30.3 mg of product **3.35** and 16.3 mg of product **3.36**.



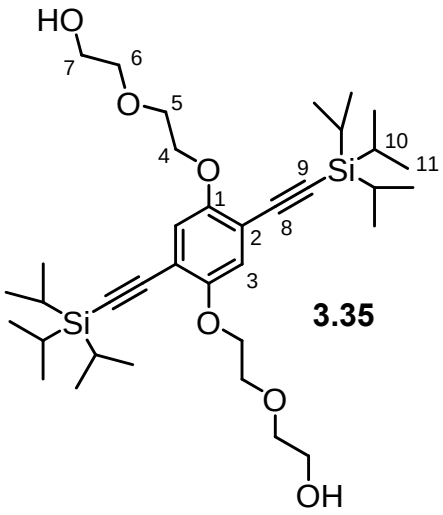
3.36

Yield: 31 %

MS (ESI) calculated for $[C_{25}H_{41}IO_6Si]^+$: 592.6; found = 593.0

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.27 (s, 1H, H_6), 6.87 (s, 1H, H_3), 4.15 – 4.05 (m, 4H, H_7, H_{11}), 3.93 – 3.78 (m, 4H, H_8, H_{12}), 3.77 – 3.58 (m, 8H, H_9, H_{10}, H_{13}, H_{14}), 1.16 – 0.86 (m, 3H, H_{17}), 1.10 - 1.12 (m, 18H, H_{18}).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 155.0 (C_1); 152.0 (C_4); 124.3 (C_6); 117.6 (C_3); 114.4 (C_2); 102.4 (C_{15}); 96.7 (C_{16}); 87.8 (C_5); 72.8, 72.8 (C_{13}, C_9); 70.1, 69.8, 69.7, 69.5 (C_7, C_8, C_{11}, C_{12}); 62.1, 62.1 (C_{10}, C_{14}); 18.9 (C_{18}); 11.5 (C_{17}).



3.35

Yield: 50 %

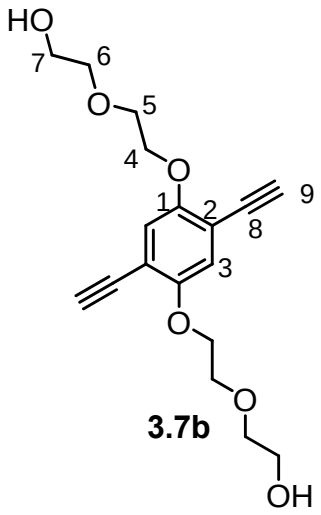
MS (ESI) calculated for $[C_{36}H_{62}O_6Si_2Na]^+$: 670.0; found = 669.8

^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.89 (s, 2H, H_3), 4.15 – 4.09 (m, 4H, H_4), 3.85 – 3.80 (m, 4H, H_5), 3.74 – 3.67 (m, 4H, H_7), 3.66 – 3.59 (m, 4H, H_6), 1.29 – 0.91 (m, 6H, H_{10}), 1.10 – 1.12 (m, 38H, H_{11}).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 154.1 (C_1); 118.2 (C_3); 114.7 (C_2); 102.9 (C_8); 97.1 (C_9); 72.7(C_6); 69.9 (C_5); 69.3 (C_4); 62.1 (C_7); 18.9 (C_{11}); 11.6 (C_{10}).

3.5.3.7 1,4-bis[2-(2-hydroxyethoxy)ethoxy]-2,5-diethynylbenzene, 3.7b

To a solution of **3.35** (200 mg, 0.31 mmol, 1 eq.), in THF (3 mL) TBAF (1 M, 0.93 mL, 0.93 mmol, 3 eq.), was added. The resulting solution was stirred for 20 min at room temperature. The solvent was removed under reduced pressure and the crude was purified by flash chromatography (silica, chloroform with gradient of methanol from 0 to 15%) to afford 62 mg of pure product.



3.7b

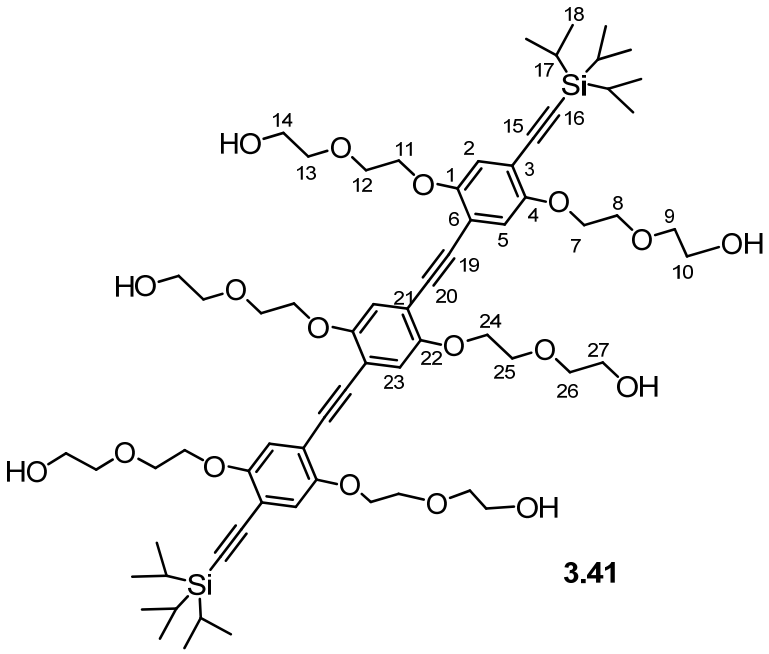
Yield: 60%

MS (ESI) calculated for $[C_{18}H_{22}NaO_6]^+$: 357.4; found = 357.3

^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.98 (s, 2H, H_3), 4.16 – 4.10 (m, 4H, H_4), 3.90 – 3.82 (m, 4H, H_5), 3.76 – 3.70 (m, 4H, H_7), 3.68 – 3.62 (m, 4H, H_6), 3.34 (s, 2H, H_9).
^{13}C NMR (100 MHz, $CDCl_3$): δ = 154.2 (C_1); 118.4 (C_3); 113.8 (C_2); 83.2 (C_9); 79.6 (C_8); 72.7 (C_6); 69.6 (C_5); 69.5 (C_4); 62.0 (C_7).

3.5.3.8 Rod 3.41 and 3.42

3.7b (50 mg, 0.151 mmol, 3 eq.), **3.36** (30 mg, 0.05 mmol, 1 eq.), $Pd(PPh_3)_4$ (5.8 mg, 0.005 mmol, 0.1 eq.), CuI (1 mg, 0.005 mmol, 0.1 eq.), and PPh_3 (2.6 mg, 0.01 mmol, 0.2 eq.) were placed into the reaction flask and dried under vacuum, then the reagents were dissolved by addition of toluene (0.6 mL) and TEA (0.2 mL) under nitrogen. The reaction was heated to 50°C overnight. The solvent was removed under reduced pressure and the resulting crude was purified by flash chromatography (chloroform with gradient of methanol from 0 to 15%) to afford 15.5 mg of product **3.41** and 5 mg of product **3.42**.



3.41

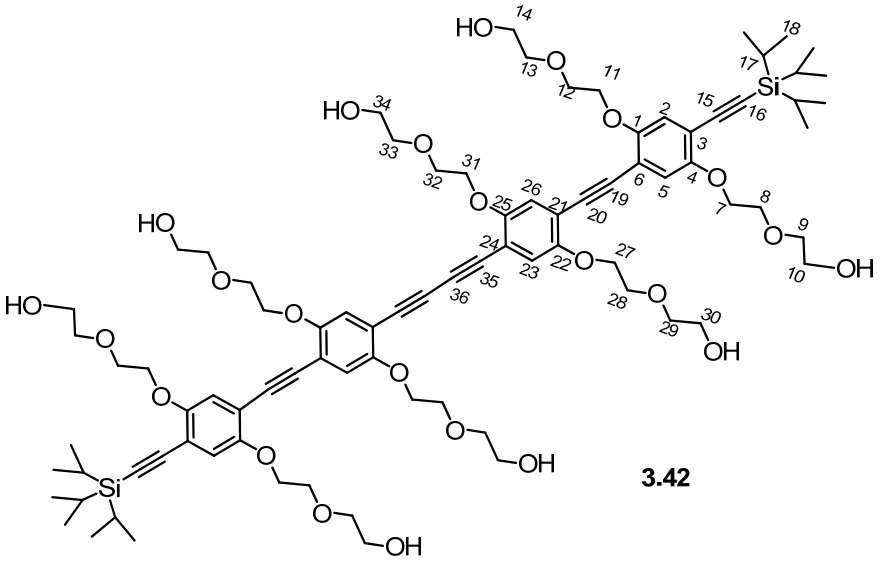
3.41

Yield: 50 %

MS (ESI) calculated for $[C_{68}H_{102}NaO_{18}Si_2]^+$: 1286.7; found = 1286.0

^{1}H NMR (400 MHz, CD_3OD): δ = 7.16 (s, 2H, H_2), 7.12 (s, 2H, H_5), 7.05 (s, 2H, H_{23}), 4.31 – 4.12 (m, 12H, H_7, H_{11}, H_{24}), 3.97 – 3.80 (m, 12H, H_8, H_{12}, H_{25}), 3.76 – 3.56 (m, 24H, H_9, H_{10}, H_{13}, H_{14}, H_{26}, H_{27}), 1.35 – 0.98 (m, 6H, H_{17}), 1.16 – 1.18 (m, 36H, H_{18}).

^{13}C NMR (100 MHz, CD_3OD): δ = 155.9, 155.2, 154.9 (C_4, C_1, C_{22}); 119.6, 119.1, 118.3 (C_5, C_2, C_{23}); 116.1, 116.0, 115.7 (C_3, C_6, C_{21}); 104.3 (C_{15}); 97.7 (C_{16}); 92.6, 92.6 (C_{19}, C_{20}); 74.3, 74.2, 74.1 (C_9, C_{13}, C_{26}); 71.2, 71.1, 71.0, 71.0, 70.5 (C_7, C_8, C_{11}, C_{12}, C_{24}, C_{25}); 65.5, 62.4 (C_{10}, C_{14}, C_{27}); 19.3 (C_{18}); 12.7 (C_{17}).



3.42

3.42

Yield: 12 %

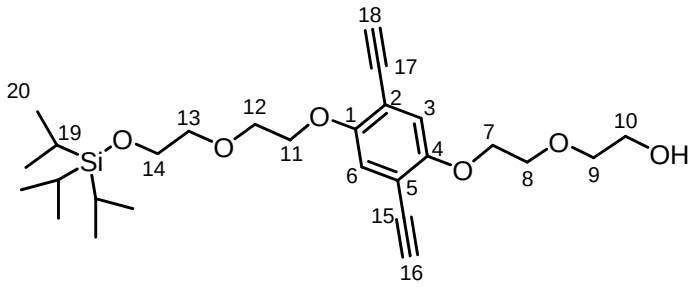
MS (ESI) calculated for $[C_{86}H_{122}O_{24}Si_2Na]^+$: 1619.0; found = 1618.9

^{1}H NMR (400 MHz, CD_3OD): δ = 7.17 (s, 2H, H_2), 7.16 (s, 2H, H_{23}), 7.12 (s, 2H, H_5), 7.05 (s, 2H, H_{26}), 4.27 – 4.15 (m, 16H, H_7, H_{11}, H_{27}, H_{31}), 3.95 – 3.82 (m, 16H, H_8, H_{12}, H_{28}, H_{32}), 3.72 – 3.59 (m, 32H, H_9, H_{10}, H_{13}, H_{14}, H_{29}, H_{30}, H_{33}, H_{34}), 1.36 – 0.96 (m, 6H, H_{17}), 1.16 – 1.18 (m, 36H, H_{18}).

^{13}C NMR (100 MHz, CD_3OD): δ = 156.7, 155.8, 154.9 (C_1, C_4, C_{22}, C_{25}); 119.7, 119.6, 118.4, 118.4 (C_2, C_5, C_{23}, C_{26}); 117.1, 116.6, 115.9, 115.6 (C_3, C_6, C_{21}, C_{24}); 74.4, 74.2, 74.2, 74.1 (C_9, C_{13}, C_{29}, C_{33}); 71.2, 71.1, 71.0, 70.9, 70.8, 70.6 (C_7, C_8, C_{11}, C_{12}, C_{27}, C_{28}, C_{31}, C_{32}); 62.6, 62.5, 62.4 (C_{10}, C_{14}, C_{30}, C_{34}); 19.3 (C_{18}); 12.7 (C_{17}).

3.5.3.9 1-[2-(2-(tris(1-methylethyl)silyloxy)ethoxy)ethoxy]-4-[2-(2-hydroxyethoxy)ethoxy]-2,5-diethynylbenzene, 3.44

To a solution of **3.35** (32 mg, 0.049 mmol, 1 eq.) in THF (0.3 mL) a solution of TBAF in THF (1 M, 0.05 mL, 0.049 mmol, 1 eq.) was added. The resulting solution was stirred for 2 h at room temperature. The solvent was removed under reduced pressure and the crude was purified by flash chromatography (silica, chloroform with gradient of methanol from 0 to 15 %) to afford 11.3 mg of product.



3.44

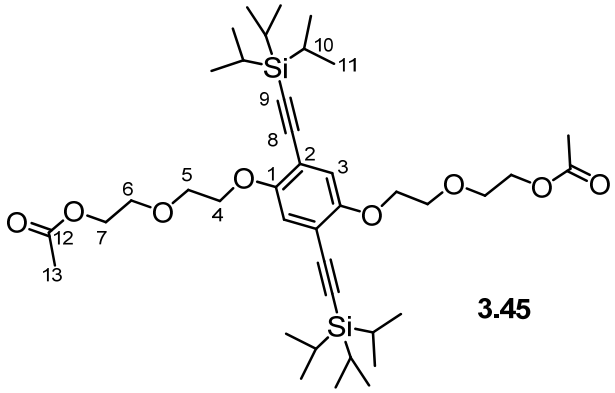
Yield: 47 %

^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.98 (s, 1H, H_3 or H_6), 6.97 (s, 1H, H_3 or H_6), 4.17 – 4.05 (m, 4H, H_7, H_{11}), 3.90 – 3.79 (m, 6H, H_8, H_{12}, H_{14}), 3.77 – 3.70 (m, 2H, H_{10}), 3.70 – 3.58 (m, 4H, H_9, H_{13}), 3.33 (s, 1H, H_{16} or H_{18}), 3.30 (s, 1H, H_{16} or H_{18}), 1.13 – 0.96 (m, 3H, H_{19}), 1.03 – 0.05 (m, 18H, H_{20}).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 154.4, 154.0 (C_1, C_4); 118.6, 118.4 (C_3 ,C_6); 113.9, 113.8 (C_2, C_5); 83.0, 83.0 (C_{16}, C_{18}); 79.7 (C_{15}, C_{17}); 73.4, 72.7 (C_9, C_{13}); 70.0, 69.8, 69.6, 69.5 (C_7, C_8, C_{11}, C_{12}); 63.3(C_{14}); 62.0 (C_{10}); 12.8 (C_{20}); 12.2 (C_{19}).

3.5.3.10 1,4-bis[2-(2-(acetoxy)ethoxy)ethoxy]-2,5-bis[tris(1-methylethyl)silyl]ethynyl)benzene, 3.45

To a solution of **3.35** (50 mg, 0.077 mmol, 1 eq.) in DCM (1 mL) Ac_2O (30 µl, 0.31 mmol, 4 eq.) and Et_3N (64 µl, 0.46 mmol, 6 eq.) were added under nitrogen atmosphere. The reaction was stirred at room temperature for 3 h. The solvent was removed under reduced pressure and the crude was purified by flash chromatography (silica, hexane with gradient of ethyl acetate from 0 to 30 %) to afford 33.2 mg of product.



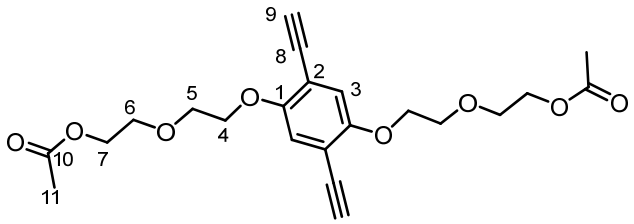
3.45

Yield: 59 %

^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.88 (s, 2H, H_3), 4.23 – 4.16 (m, 4H, H_7), 4.14 – 4.05 (m, 4H, H_4), 3.86 – 3.79 (m, 4H, H_5), 3.78 – 3.68 (m, 4H, H_6), 2.05 (s, 6H, H_{13}), 1.27 – 0.89 (m, 6H, H_{10}), 1.10 – 1.12 (m, 36H, H_{11}).

3.5.3.11 1,4-bis[2-(2-(acetoxy)ethoxy)ethoxy]-2,5-diethynylbenzene, 3.46

To a solution of **3.45** (33 mg, 0.045 mmol, 1 eq.) in THF (1 mL) a solution of TBAF in THF (0.03 M, 0.3 mL, 0.009 mmol, 0.2 eq.), was added. The resulting solution was stirred for 10 min at room temperature. The solvent was removed under reduced pressure and the crude was purified by flash chromatography (silica, hexane with gradient of ethyl acetate from 0% to 60%) to afford 20.1 mg of product.



3.46

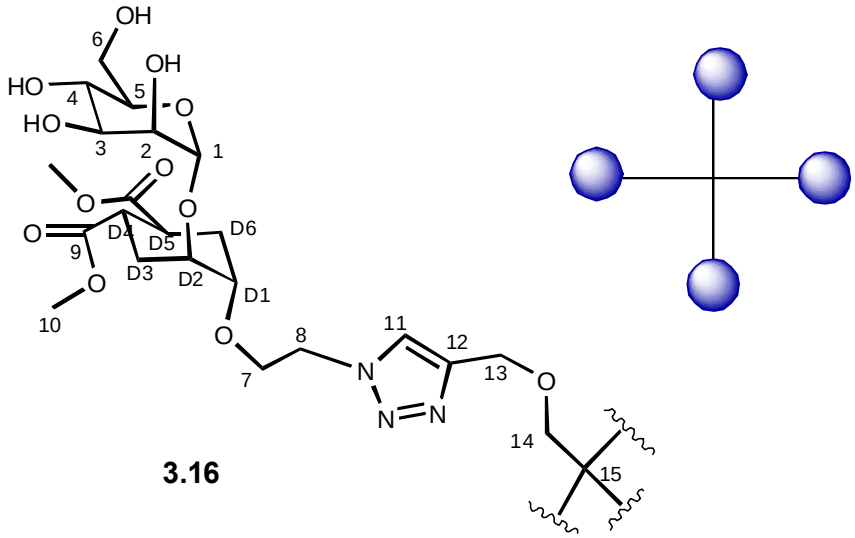
Yield: 97%

^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.97 (s, 2H, H_3), 4.24 – 4.18 (m, 4H, H_7), 4.16 – 4.08 (m, 4H, H_4), 3.89 – 3.82 (m, 4H, H_5), 3.81 – 3.75 (m, 4H, H_6), 3.31 (s, 2H, H_9), 2.06 (s, 6H, H_{11}).

3.5.4 Synthesis of glycodendrons and glycodendriners 3.15–3.21, 3.23-3.26 and 3.28

3.5.4.1 Tetravalent glycodendrimer 3.16

Pseudodisaccharide **1.7b**[16] (10 mg, 0.02 mmol, 4.4 eq.), scaffold **3.2** (1.4 mg, 0.005 mmol, 1 eq.), copper(II) sulphate pentahydrate (0.72 mg, 0.003 mmol, 0.1 eq.), sodium ascorbate (2.3 mg , 0.012 mmol, 0.4 eq.) and TBTA (3 mg, 0.006 mmol, 0.2 eq.) were dissolved in 0.6 mL of THF/H_2O (1:1). After 2.5 h, the solvent was removed under reduced pressure and the resulting crude was purified by size exclusion chromatography (Sephadex LH20, MeOH) to afford 8.6 mg of pure product.



3.16

Yield: 85 %

$[\alpha]_D^{25}$ = + 26 (c = 0.2, MeOH)

MS (ESI-HRMS) calculated for $[C_{89}H_{136}N_{12}O_{48}Na]^+$: 2163.8468; found = 2163.8449

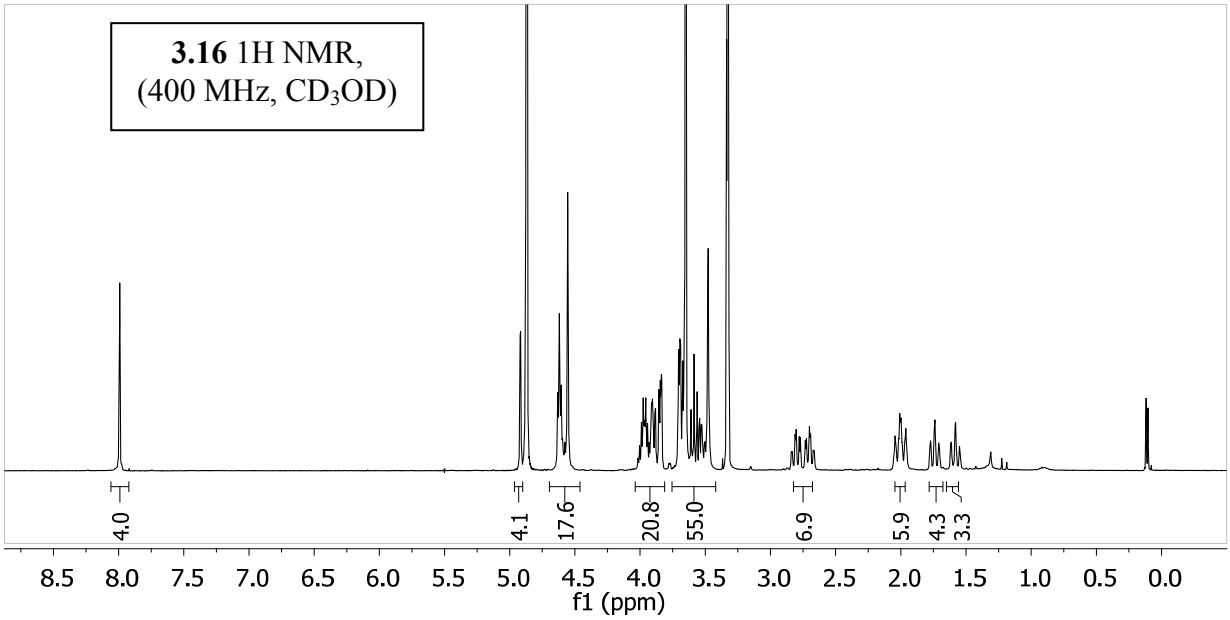
MS (MALDI, matrix: sinapinic acid, solvent H_2O/MeOH)

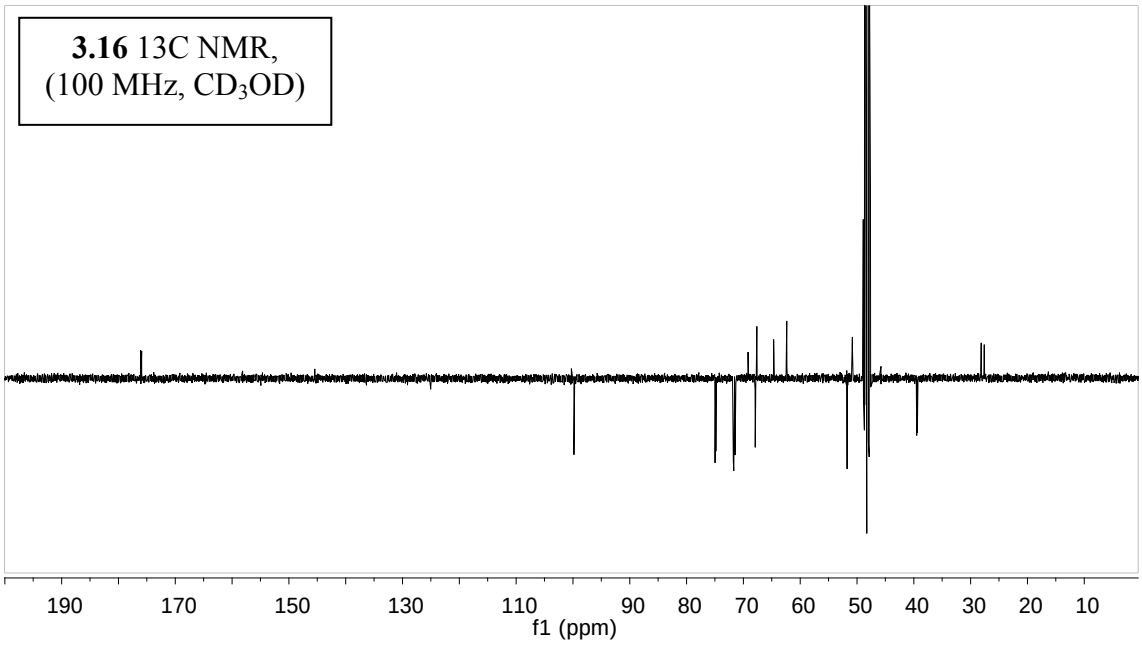
calculated for $[C_{89}H_{136}N_{12}O_{48}]^+$: 2142.1; found = 2142.8

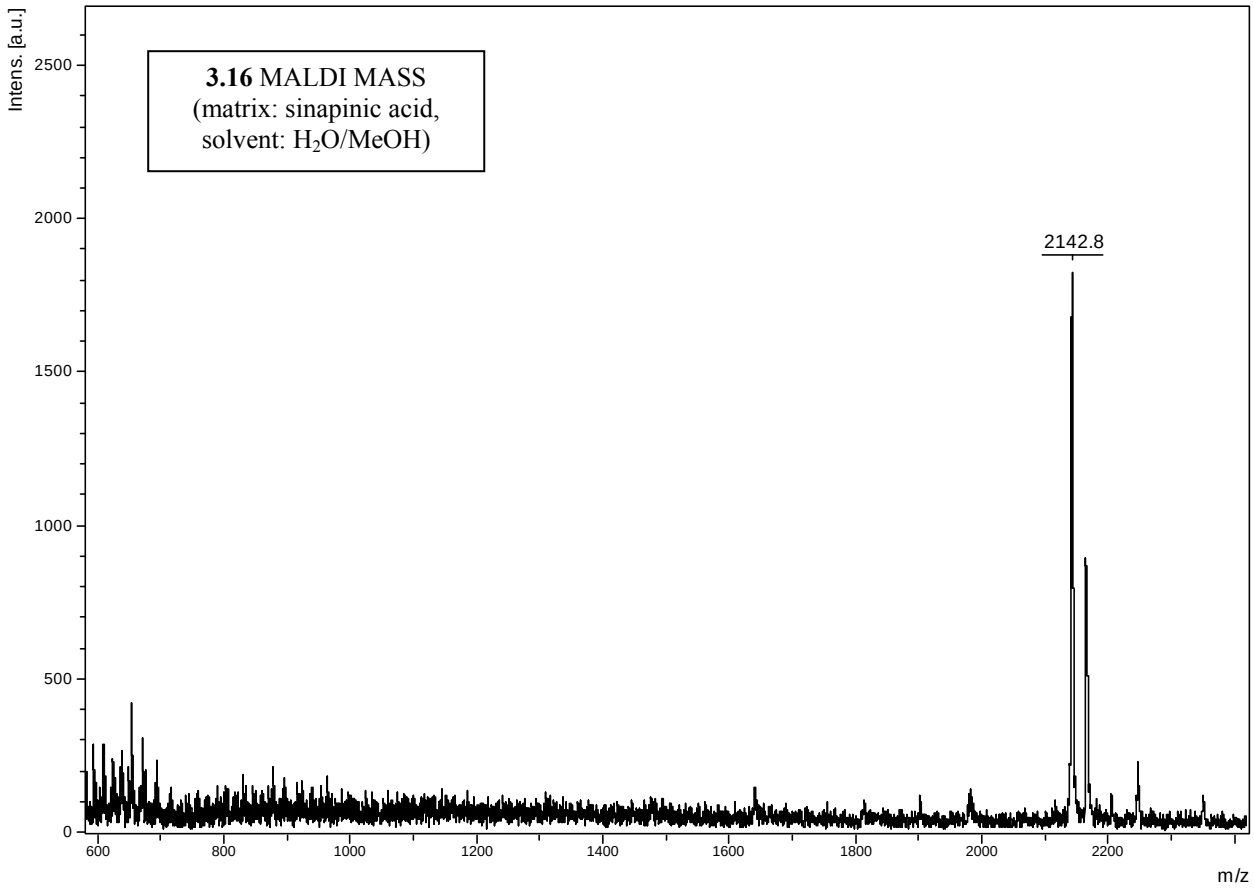
calculated for $[C_{89}H_{136}N_{12}O_{48}Na]^+$: 2165.1; found = 2165.1

^{1}H NMR (400 MHz, D_2O): δ = 8.00 (s, 4H, H_{11}), 4.96 (br s, 4H, H_1), 4.63 - 4.59 (m, 8H, H_8), 4.56 (m, 8H, H_{13}), 3.98 - 3.94 (m, 12H, H_2, H_7), 3.89 - 3.85 (m, 8H, H_{6a}, D_2), 3.81 (m, 4H, H_3), 3.77 - 3.65 (m, 32H, H_{6b}, D_1, H_{10}), 3.65 - 3.58 (m, 8H, H_4, H_5), 3.42 (s, 8H, H_{14}), 2.84 (td, 4H, D_4 or D_5, J = 12.3 Hz, 3.2 Hz), 2.42 (td, 4H, D_4 or D_5, J = 12 Hz, 2.8 Hz), (td, J = 12 Hz, 2.8 Hz, 4 Hz), 2.03-1.94 (m, 8H, $D_{3eq.}$, $D_{6eq.}$), 1.77 - 1.70 (m, 4H, D_{6ax}), 1.48-1.41 (m, 4H, D_{3ax})

^{13}C NMR (100 MHz, D_2O): δ = 177.2, 176.8 (C_9); 144.4 (C_{12}); 125.2 (C_{11}); 98.6 (C_1); 73.6 (D_1); 73.4 (C_4); 70.8 (D_2); 70.5, 70.4 (C_2, C_3); 68.1 (C_{14}); 66.7 (C_7); 66.5 (C_5); 63.7 (C_{13}); 60.9 (C_6); 52.5 (C_{10}); 50.4 (C_8); 44.8 (C_{15}); 38.7 (D_4, D_5); 26.8, 26.5 (D_3, D_6).

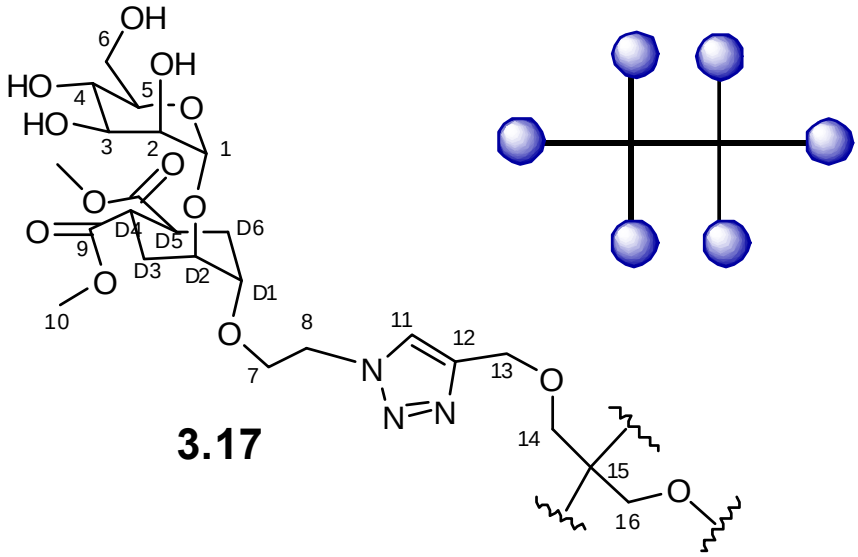

3.16 1H NMR,
(400 MHz, CD_3OD)
4.0
4.1
17.6
20.8
55.0
6.9
5.9
4.3
3.3
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


3.16 13C NMR,
(100 MHz, CD_3OD)
190 170 150 130 110 90 80 70 60 50 40 30 20 10
f1 (ppm)



3.5.4.2 Hexavalent glycodendrimer 3.17

Pseudodisaccharide **1.7b**[16] (20 mg, 0.04 mmol, 6.6 eq.), scaffold **3.3** (2.3 mg, 0.005 mmol, 1 eq.), copper(II) sulphate pentahydrate (0.48 mg, 0.002 mmol, 0.1 eq.), sodium ascorbate (1.6 mg , 0.048 mmol, 0.4 eq.) and TBTA (1 mg, 0.002 mmol, 0.2 eq.) were dissolved in 1 mL of THF/H_2O (1:1). After 4 h, the solvent was removed under reduced pressure and the resulting crude was purified by size exclusion chromatography (Sephadex LH20, MeOH) to afford 12 mg of pure product.



3.17

Yield: 79 %

$[\alpha]_D^{25}$ = + 30 (c = 0.4, MeOH)

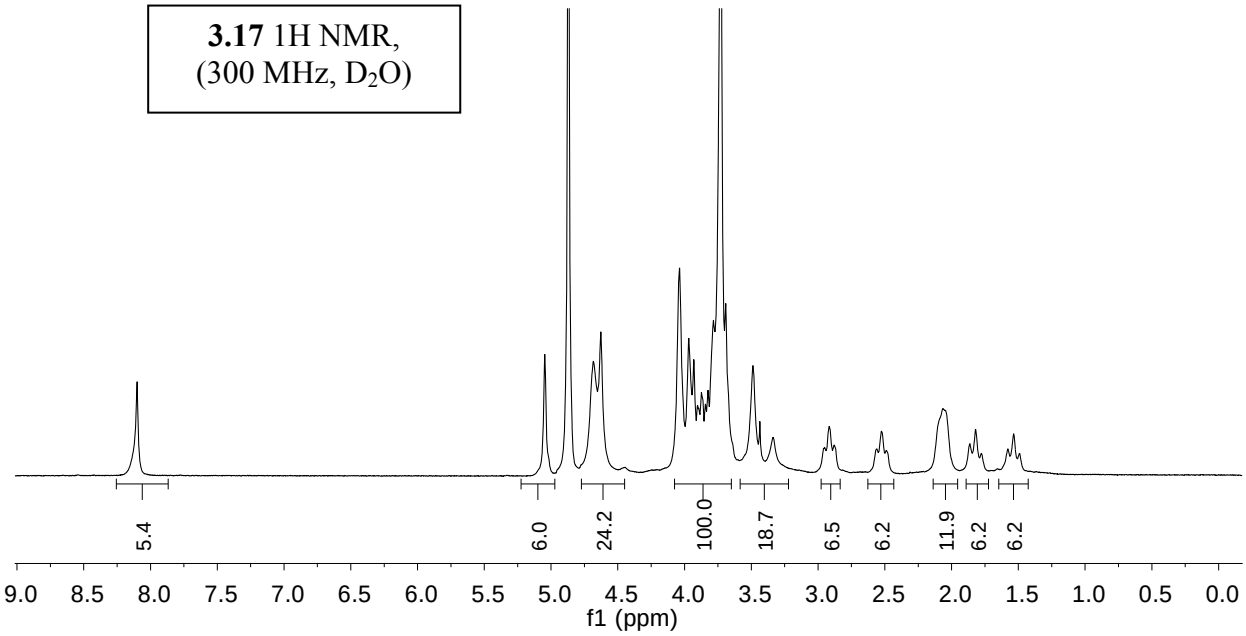
MS (ESI-HRMS) calculated for $[C_{136}H_{208}N_{18}O_{73}Na]^{++}$: 1653.6450; found = 1653.6460

MS (ESI) calculated for $[C_{136}H_{208}N_{18}O_{73}Na]^{+}$: 3286.2; found = 3285.3

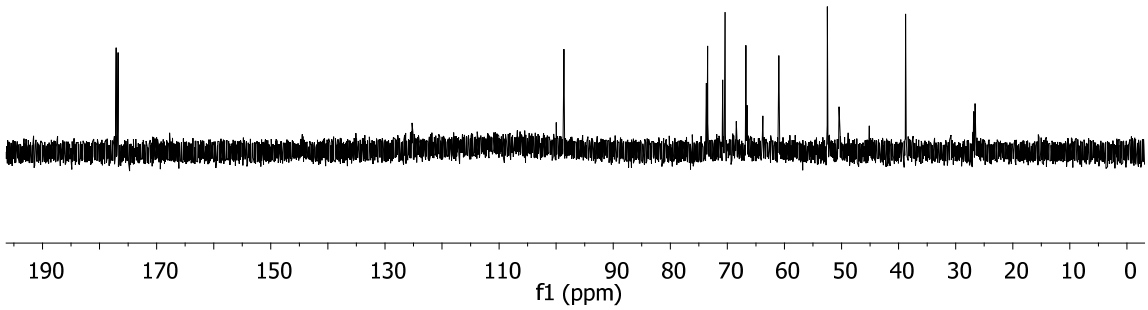
^{1}H NMR (400 MHz, D_2O): δ = 8.01 (s, 6H, H_{11}), 4.98 (br s, 6H, H_1), 4.60 - 4.56 (m, 24H, H_{13}, H_8), 3.98 - 3.92 (m, 18H, H_2, H_7), 3.90 - 3.47 (m, 78H, H_{6ab}, D_2, H_3, D_1, H_{10}, H_4, H_5), 3.42 (s, 12H, H_{14}), 3.19 (s, 4H, H_{16}), 2.88 – 2.81 (m, 6H, D_4), 2.47 – 2.41 (m, 6H, D_5), 1.94 – 1.85 (m, 12H, $D_{3eq.}$, $D_{6eq.}$), 1.78 - 1.70 (m, 6H, D_{6ax}), 1.49 - 1.41 (m, 6H, D_{3ax}).

^{13}C NMR (100 MHz, D_2O): δ = 177.1, 176.8 (C_9); 144.3 (C_{12}); 125.3 (C_{11}); 98.6 (C_1); 73.6 (D_1); 73.5 (C_4); 70.8 (D_2); 70.5, 70.4 (C_2, C_3); 68.1 (C_{14}); 66.7 (C_7); 66.6 (C_5); 63.8 (C_{13}); 60.9 (C_6); 52.4 (C_{10}); 50.4 (C_8); 45.1 (C_{15}); 38.7 (D_4, D_5); 26.9, 26.6 (D_3, D_6).

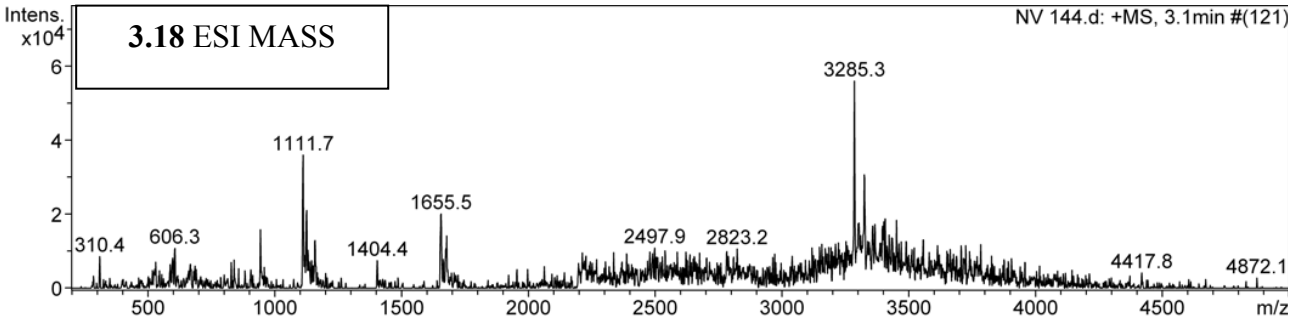
3.17 1H NMR,
(300 MHz, D_2O)



3.17 1H NMR,
(300 MHz, D_2O)

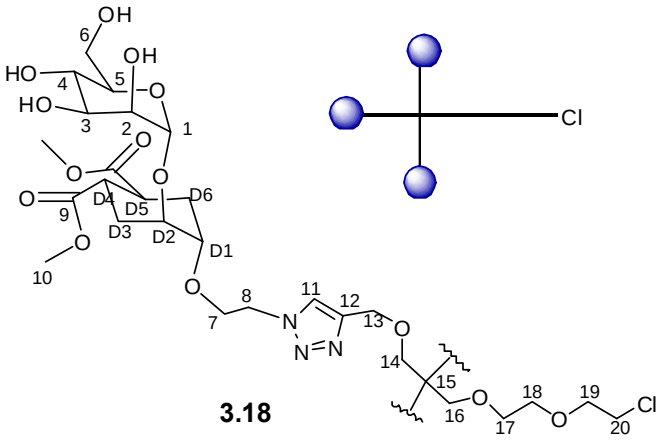


3.18 ESI MASS



3.5.4.3 Trivalent glycodendron 3.18

Pseudodisaccharide **1.7b**[16] (210 mg, 0.453 mmol, 3.3 eq.), scaffold **3.5** (46.2 mg, 0.129 mmol, 1 eq.), copper(II) sulphate pentahydrate (3.4 mg, 0.0136 mmol, 0.1 eq.), sodium ascorbate (10.8 mg , 0.054 mmol, 0.4 eq.) and TBTA (14 mg, 0.026 mmol, 0.4 eq.) were dissolved in 10 mL of THF/H_2O (1:1). After 1 h TLC (hex:EA = 8:0) indicated presence of scaffold **3.5**, therefore another portion of sodium ascorbate (10.8 mg , 0.054 mmol, 0.4 eq.) was added, and the reaction was stirred another 4 h. The solvent was removed under reduced pressure and the resulting crude was purified by size exclusion chromatography (Sephadex LH20, MeOH) to afford 198 mg of pure product.



3.18

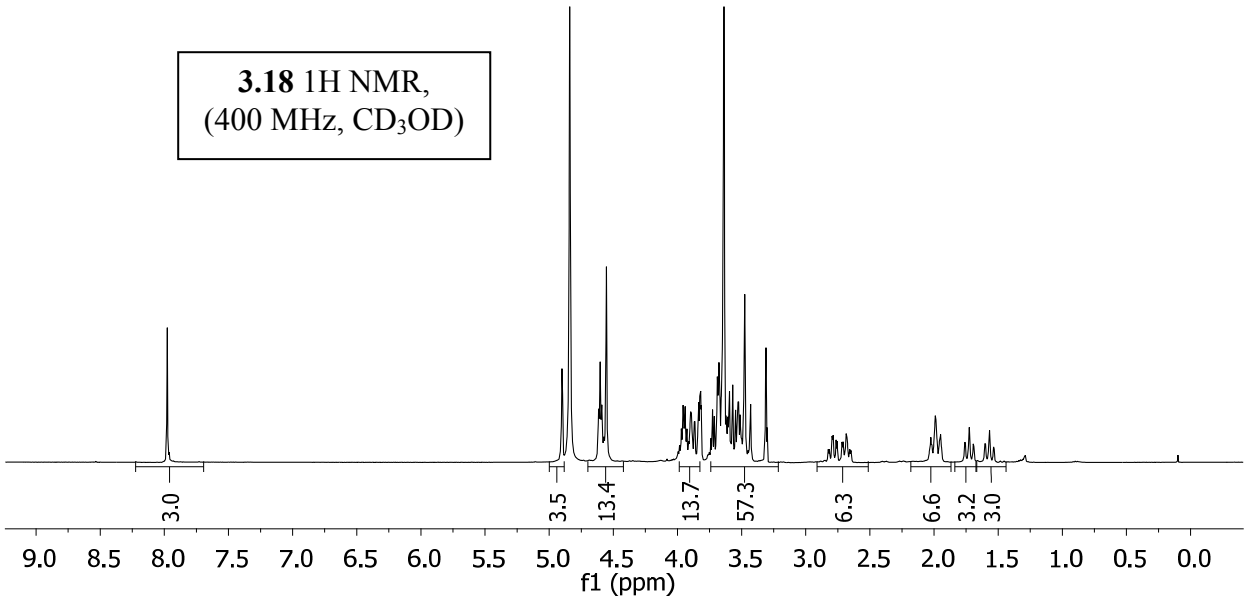
Yield: 88 %

MS (ESI) calculated for $[C_{72}H_{112}ClN_9O_{38}Na]^+$: 1768.7; found = 1768.7

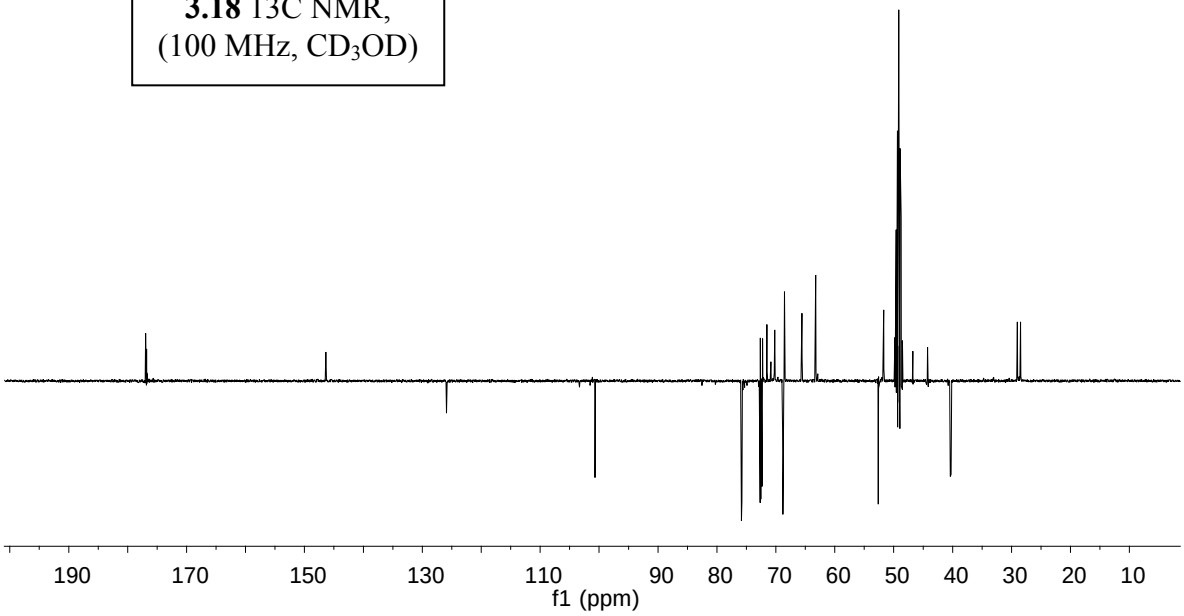
^{1}H NMR (400 MHz, CD_3OD): δ = 7.98 (s, 3H, H_{11}), 4.90 (br s, 3H, H_1), 4.60 (t, 6H, H_8, $J_{8\text{-}7}$ = 5 Hz), 4.55 (s, 6H, H_{13}), 4.00 - 3.79 (m, 15H, H_2, H_{6a}, D_2, H_7), 3.75 - 3.45 (m, 37H, H_{6b}, D_1, H_3, H_{10}, H_4, H_5, H_{17}, H_{18}, H_{19}, H_{20}), 3.48 (s, 6H, H_{14}), 3.43 (s, 2H, H_{16}), 2.83 – 2.63 (m, 6H, D_4, D_5), 2.04 – 1.92 (m, 6H, $D_{3eq.}$, $D_{6eq.}$), 1.78 - 1.51 (m, 6H, D_{3ax}, D_{6ax}).

^{13}C NMR (100 MHz, CD_3OD): δ = 177.0, 176.8 (C_9); 146.4 (C_{12}); 125.9 (C_{11}); 100.7 (C_1); 75.8 (C_3); 75.8 (C_5); 72.7 (C_{D1}); 72.7 (C_{19}); 72.6 (C_2); 72.3 (D_2); 72.2 (C_{17}); 71.5 (C_{16}); 70.8 (C_{14}); 70.2 (C_{18}); 68.8 (C_4); 68.5 (C_7); 65.6 (C_{13}); 63.2 (C_6); 52.6 (C_{10}); 51.7 (C_8); 46.8 (C_{15}); 44.2 (C_{20}); 40.4, 40.3 (C_{D4}, C_{D5}); 29.0, 28.5 (C_{D3}, C_{D6}).

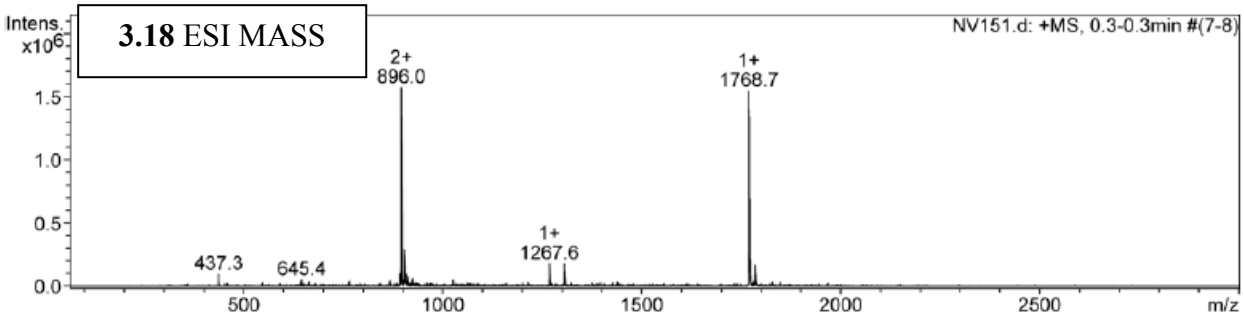
3.18 1H NMR,
(400 MHz, CD_3OD)



3.18 13C NMR,
(100 MHz, CD_3OD)

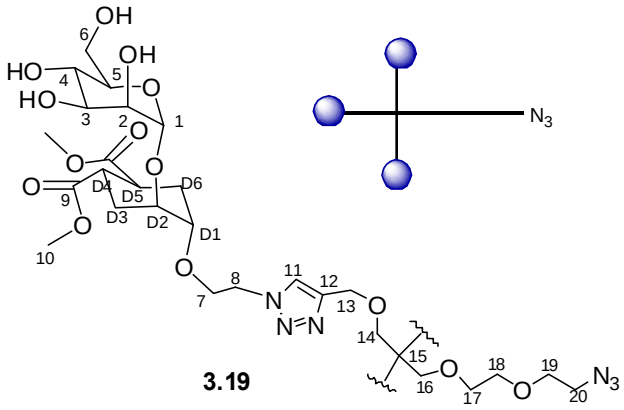


3.18 ESI MASS



3.5.4.4 Trivalent glycodendron 3.19

To a solution of **3.18** (150 mg, 0.0855 mmol, 1 eq.) in DMF (1 mL) sodium azide (44.5 mg, 0.684 mmol, 8 eq.) was added. The reaction was stirred at 60ºC for 4 days. The solvent was removed under reduced pressure and the resulting crude was purified by size exclusion chromatography (Sephadex LH20, MeOH) to afford 130 mg of pure product.



3.19

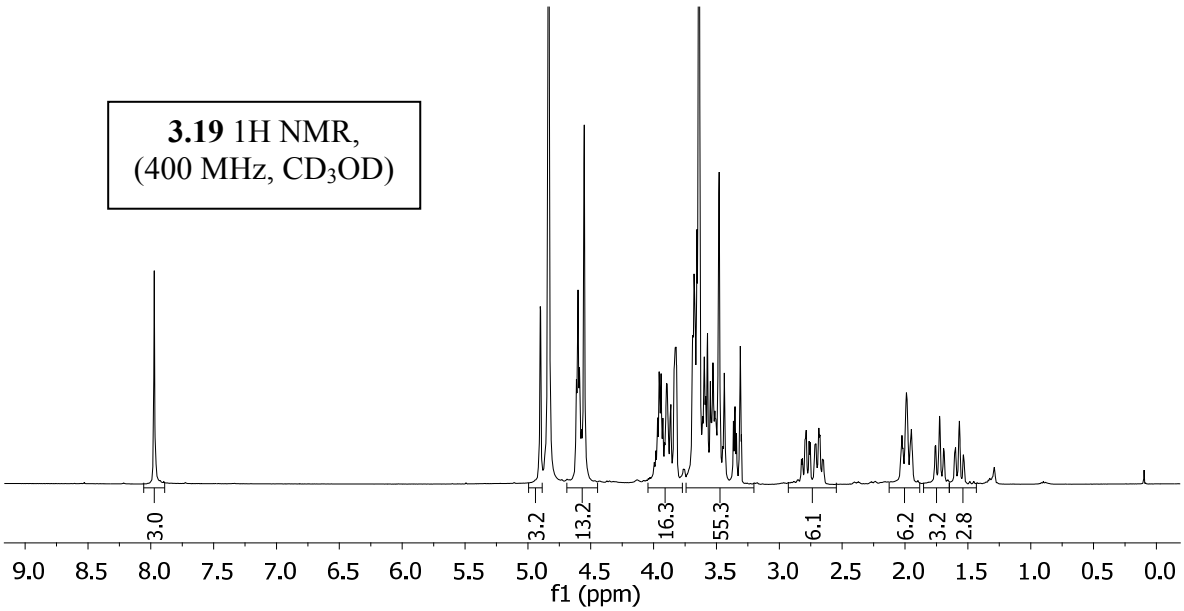
Yield: 87 %

MS (ESI) calculated for $[C_{72}H_{112}N_{12}O_{38}Na]^+$: 1775.5; found = 1775.5

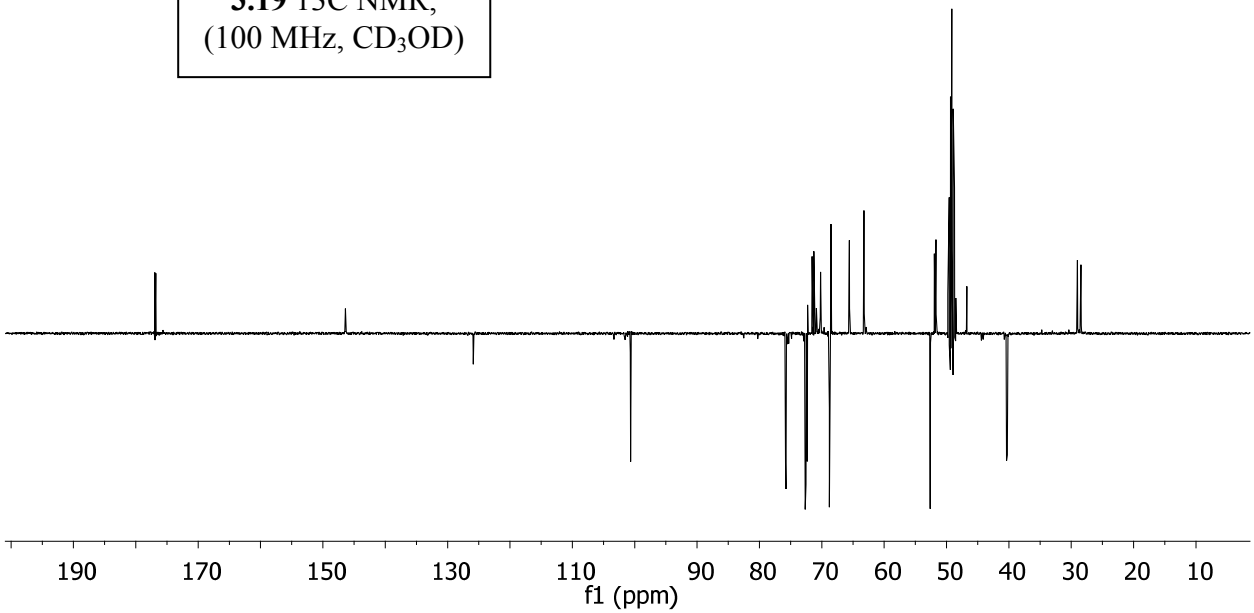
^{1}H NMR (400 MHz, CD_3OD): δ = 7.97 (s, 3H, H_{11}), 4.90 (br s, 3H, H_1), 4.60 (t, 6H, H_8, $J_{8\text{-}7}$ = 5 Hz), 4.55 (s, 6H, H_{13}), 4.01 - 3.80 (m, 15H, H_2, H_{6a}, D_2, H_7), 3.72 - 3.49 (m, 35H, H_{6b}, D_1, H_3, H_{10}, H_4, H_5, H_{17}, H_{18}, H_{19}), 3.48 (s, 6H, H_{14}), 3.44 (s, 2H, H_{16}), 3.40 – 3.32 (m, 2H, H_{20}), 2.84 – 2.61 (m, 6H, D_4, D_5), 2.05 – 1.95 (m, 6H, $D_{3eq.}$, $D_{6eq.}$), 1.77 - 1.46 (m, 6H, D_{3ax}, D_{6ax}).

^{13}C NMR (100 MHz, CD_3OD): δ = 177.0, 176.8 (C_9); 146.4 (C_{12}); 125.9 (C_{11}); 100.7 (C_1); 75.8 (C_3); 75.7 (C_5); 72.7 (C_{D1}); 72.5 (C_2); 72.3 (D_2); 72.3 (C_{17}); 71.6 (C_{16}); 71.3 (C_{19}); 70.9 (C_{14}); 70.2 (C_{18}); 68.8 (C_4); 68.5 (C_7); 65.6 (C_{13}); 63.2 (C_6); 52.6 (C_{10}); 52.0 (C_{20}); 51.7 (C_8); 46.8 (C_{15}); 40.4, 40.2 (C_{D4}, C_{D5}); 29.0, 28.5 (C_{D3}, C_{D6}).

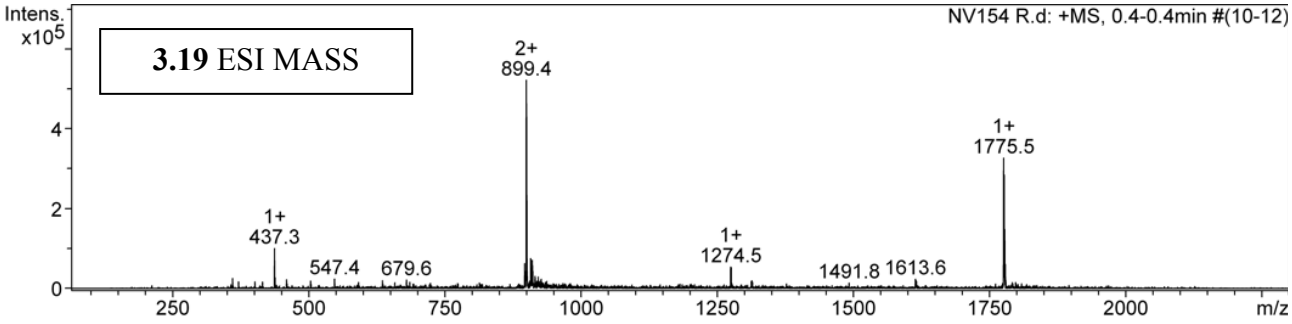
3.19 1H NMR,
(400 MHz, CD_3OD)



3.19 13C NMR,
(100 MHz, CD_3OD)

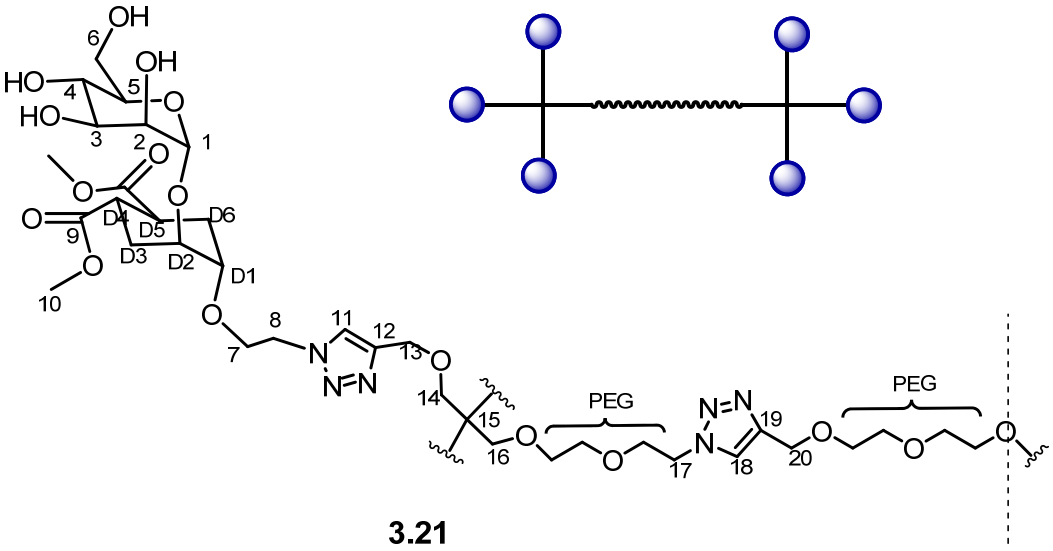


3.19 ESI MASS



3.5.4.5 Hexavalent glycodendrimer 3.21

Glycodendron **3.19** (25 mg, 0.0142 mmol, 2.2 eq.), bis-alkyne **3.4** (1.7 mg, 0.00648 mmol, 1 eq.), copper(II) sulphate pentahydrate (0.16 mg, 0.0006 mmol, 0.1 eq.), sodium ascorbate (0.51 mg , 0.0025 mmol, 0.4 eq.) and TBTA (0.68 mg, 0.0013 mmol, 0.2 eq.) were dissolved in 1 mL of THF/H_2O (1:1). After 2 h TLC (hex:EA = 8:2) indicated presence of bys-alkyne **3.4**, therefore another portion of sodium ascorbate (0.51 mg , 0.0025 mmol, 0.4 eq.) was added, and the reaction was stirred overnight. The solvent was removed under reduced pressure and the resulting crude was purified twice by size exclusion chromatography (Sephadex LH20, MeOH) to afford 11.3 mg of product.



3.21

Yield: 54 %

$[\alpha]_D^{25}$ = 17 (c = 0.22, MeOH)

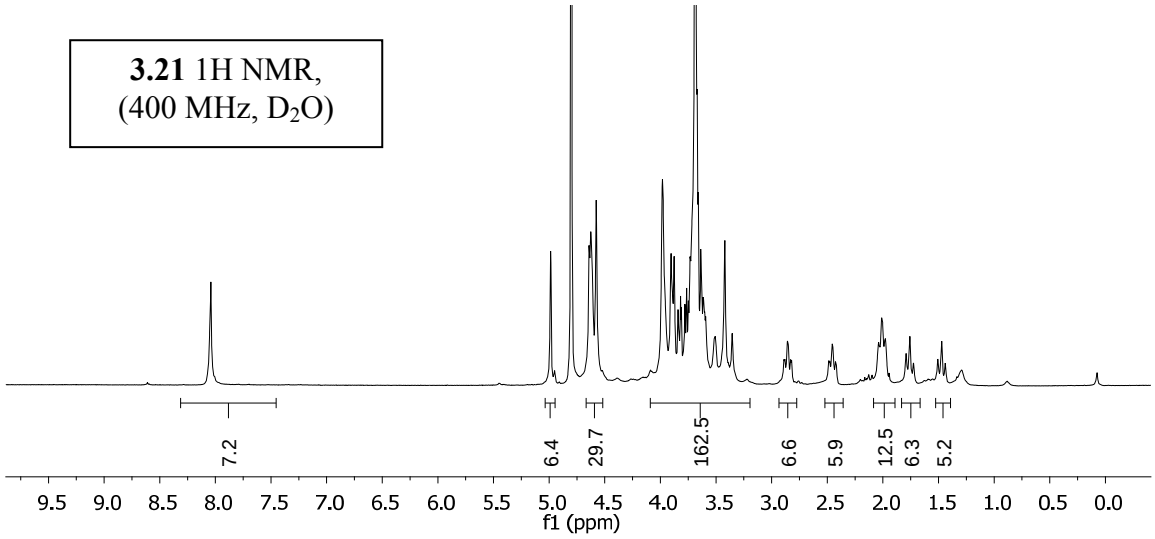
MS (ESI) calculated for $[C_{158}H_{246}N_{24}O_{81}Na]^+$: 3798.6; found = 3799.4

^{1}H NMR (400 MHz, D_2O): δ = 8.04 (br s, 8H, H_{11}, H_{18}), 4.99 (br s, 6H, H_1), 4.68 - 4.52 (m, 24H, H_{13}, H_8), 3.98 - 3.92 (m, 18H, H_2, H_7), 4.04 - 3.93 (m, 18H, H_{6a}, D_2), 3.83 (dd, 6H, H_3, $J_{3\text{-}2}$ = 3.2 Hz, $J_{3\text{-}4}$ = 9.0), 3.80 - 3.47 (m, 96H, H_{10}, H_5, H_4, H_{6b}, D_1, H_{17}, H_{20}, H_{PEG}), 3.42 (s, 12H, H_{14}), 3.35 (s, 4H, H_{16}), 2.90 – 2.80 (m, 6H, D_4), 2.50 – 2.41 (m, 6H, D_5), 2.06 – 1.93 (m, 12H, $D_{3eq.}$, $D_{6eq.}$), 1.81 - 1.70 (m, 6H, D_{6ax}), 1.53 - 1.41 (m, 6H, D_{3ax}).

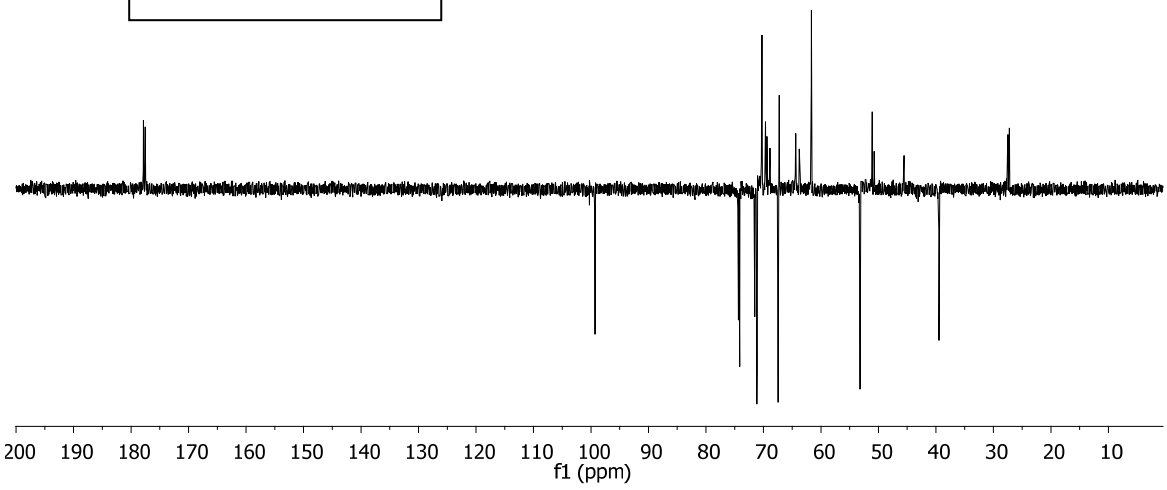
^{13}C NMR (100 MHz, D_2O): δ = 177.9, 177.6 (C_9); 125.4 (C_{11}); 99.3 (C_1); 74.3 (D_1); 73.5 (C_5); 71.5 (D_2); 71.1, 71.1 (C_2, C_3); 70.3, 70.3, 70.2, 69.7, 69.4, 68.9 (C_{14}, C_{16}, C_{PEG}); 67.4 (C_4); 67.3

(C_7); 64.4, 64.4 (C_{13}, C_{20}); 61.7 (C_6); 53.2, 53.2 (C_{10}); 51.1, 50.7 (C_{17}, C_8); 45.5 (C_{15}); 39.5, 39.4 (C_4, C_5); 27.5, 27.2 (D_3, D_6).

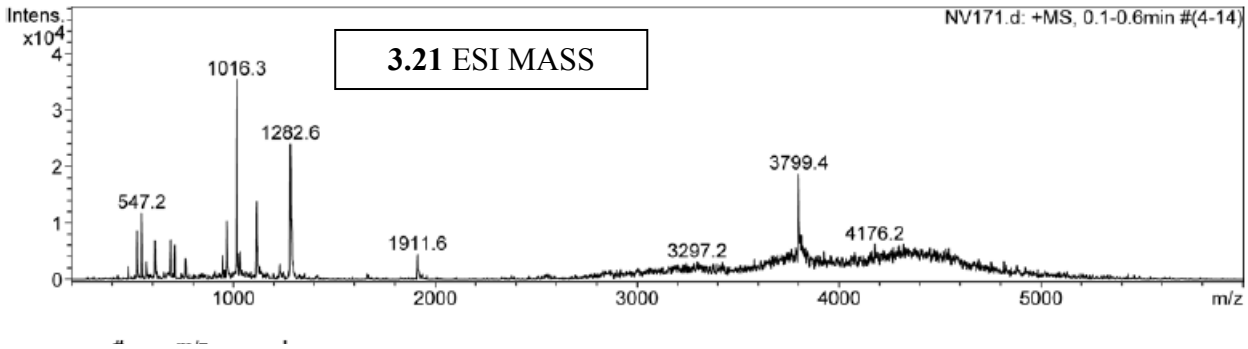
3.21 1H NMR, (400 MHz, D_2O)



3.21 13C NMR, (100 MHz, D_2O)

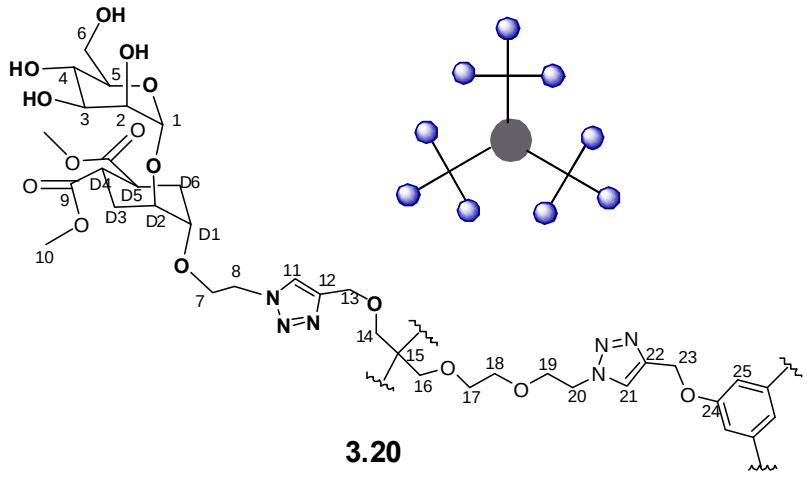


3.21 ESI MASS



3.5.4.6 Nonavalent glycodendrimer 3.20

Glycodendron **3.19** (20 mg, 0.0114 mmol, 3.3 eq.), tris-alkyne **3.1** (0.83 mg, 0.00345 mmol, 1 eq.), copper(II) sulphate pentahydrate (0.086 mg, 0.0003 mmol, 0.1 eq.), sodium ascorbate (0.27 mg , 0.0014 mmol, 0.4 eq.) and TBTA (0.36 mg, 0.0007 mmol, 0.2 eq.) were dissolved in 1 mL of THF/H_2O (1:1). After 2 h TLC (C18, H_2O:MeOH = 1:1) indicated presence of several products (probably intermediates), therefore another portion of sodium ascorbate (0.27 mg , 0.0014 mmol, 0.4 eq.) was added, and the reaction was stirred overnight. The solvent was removed under reduced pressure and the resulting crude was purified by size exclusion chromatography (Sephadex LH20, MeOH) and reverse phase flash chromatography (C18, water with gradient of MeOH from 30 to 50%) to afford 13 mg of product.



3.20

Yield: 54 %

$[\alpha]_D^{25}$ = 22 (c = 0.22, MeOH)

MS (MALDI, matrix: 2,5-dihydroxybenzoic acid, solvent: MeOH)

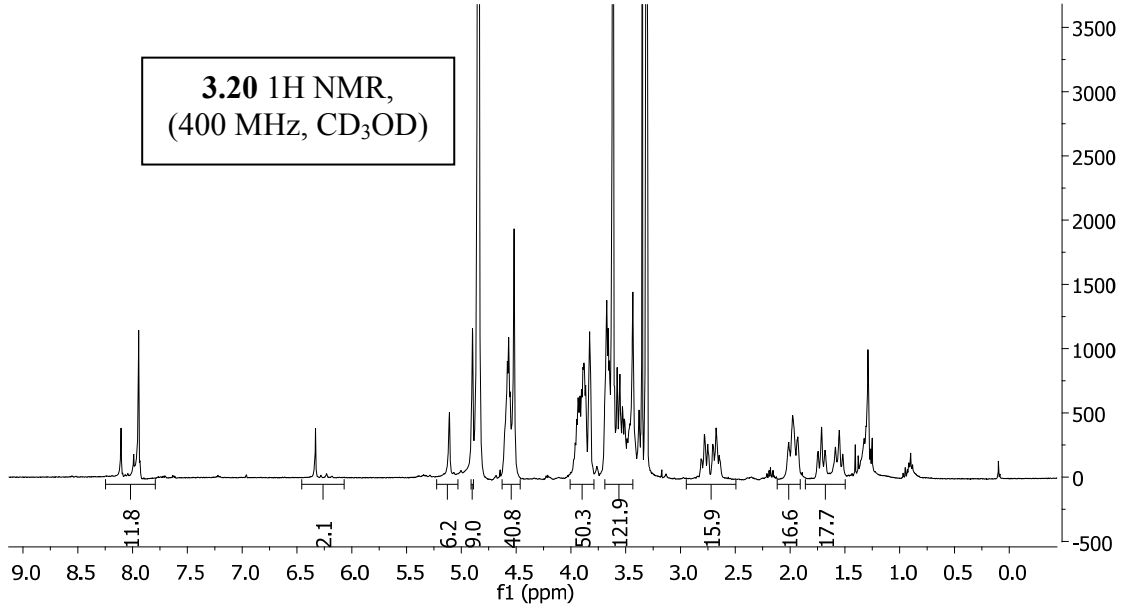
calculated for $[C_{231}H_{348}N_{36}O_{117}]^+$: 5501.4; found = 5502.5

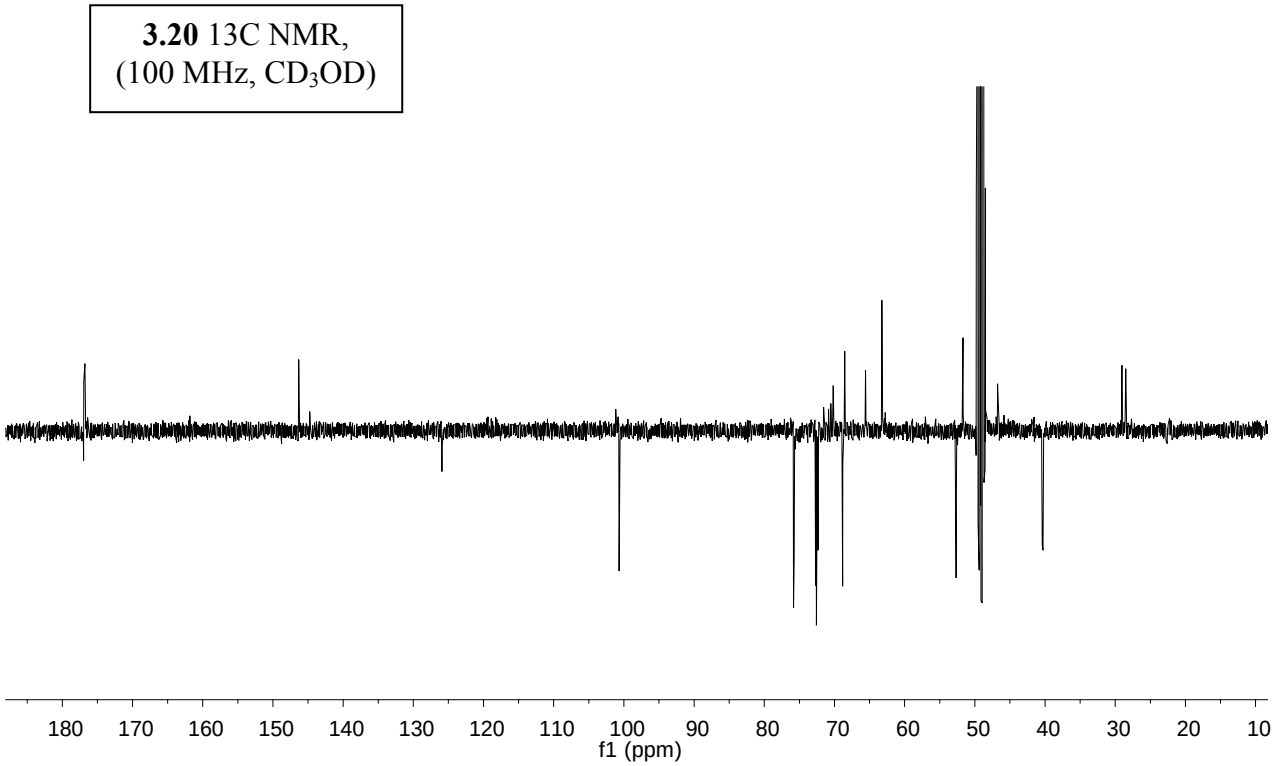
calculated for $[C_{231}H_{348}N_{36}O_{117}Na]^+$: 5524.4; found = 5525.7

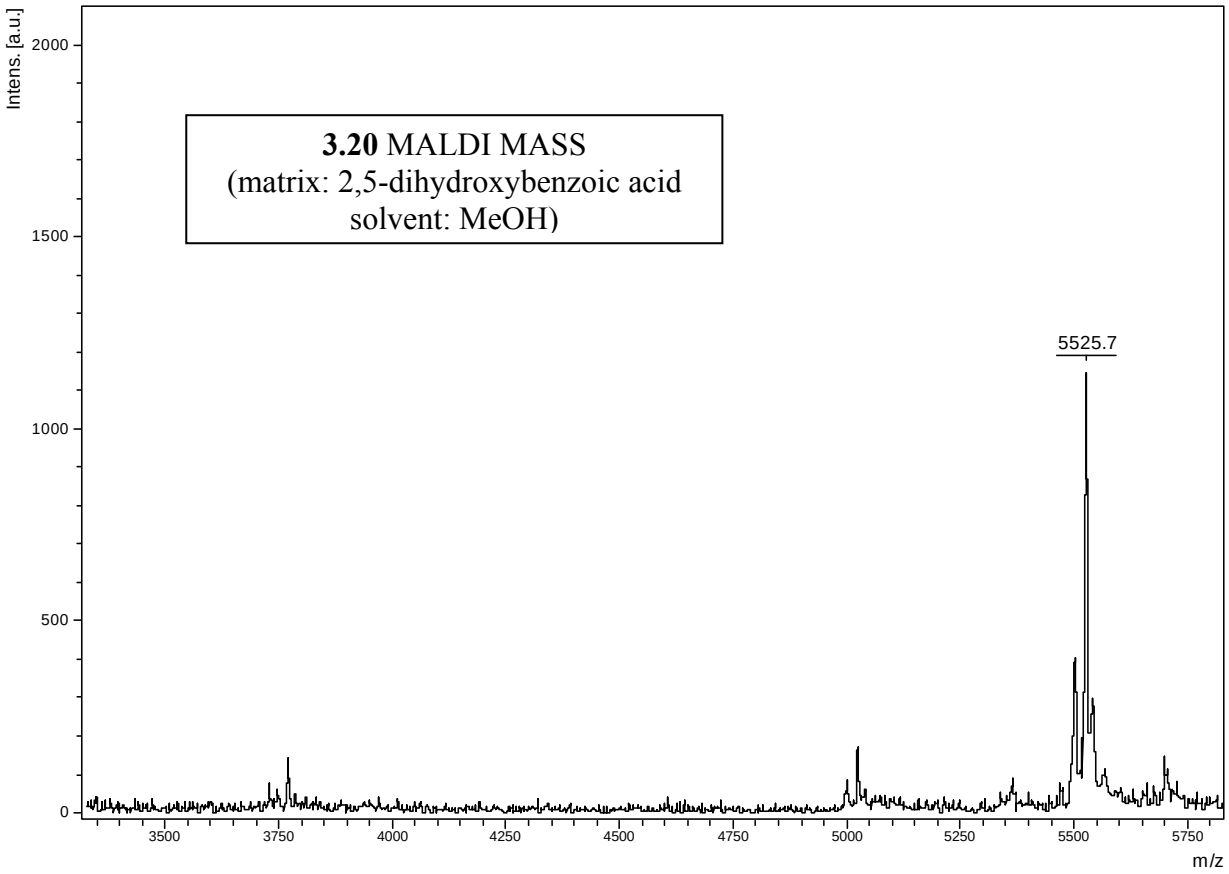
^{1}H NMR (400 MHz, CD_3OD): δ = 8.10 (s, 3H, H_{21}), 7.94 (s, 9H, H_{11}), 6.33 (s, 3H, H_{25}), 5.11 (s, 6H, H_{23}), 4.90 (br s, 9H, H_1), 4.62 – 4.49 (m, 42H, H_8, H_{13}, H_{20}), 3.99 – 3.78 (m, 45H, H_7, H_2, H_{6a}, D_2), 3.71 - 3.46 (m, 117H, H_{6b}, D_1, H_3, H_{10}, H_4, H_5, H_{17}, H_{18}, H_{19}), 3.44 (s, 18H, H_{14}), 3.38 (s, 6H, H_{16}), 2.83 – 2.58 (m, 18H, D_4, D_5), 2.05 – 1.90 (m, 18H, $D_{3eq.}$, $D_{6eq.}$), 1.77 - 1.49 (m, 18H, D_{3ax}, D_{6ax}).

^{13}C NMR (100 MHz, CD_3OD): δ = 177.0, 176.8 (C_9); 146.3 (C_{12}); 144.8 (C_{22}); 126.4 (C_{21}); 125.9 (C_{11}); 100.7 (C_1); 96.6 (C_{24}); 75.8, 75.8 (C_5, C_3); 72.7 (C_{D1}); 72.6 (C_2); 72.3 (D_2); 72.2,

71.5, 70.8, 70.5, 70.2 (C_{14}, C_{16}, C_{17}, C_{19}, C_{18}); 68.8 (C_4); 68.5 (C_7); 65.6 (C_{13}); 63.2 (C_{23}, C_6); 52.7 (C_{10}); 51.7 (C_8); 46.8 (C_{15}); 40.4, 40.3 (C_{D4}, C_{D5}); 29.0, 28.5 (C_{D3}, C_{D6}).

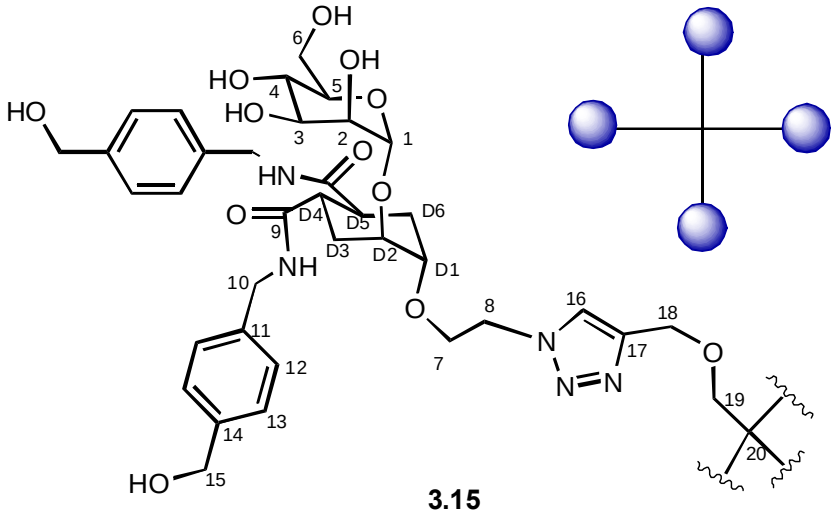






3.5.4.7 Tetravalent glycodendrimer 3.15

A flask was charged with the following reagents in the following order: tetra-alkyne **3.2** (1.94 mg, 0.00674 mmol, 1 eq.), TBTA (3.6 mg, 0.0067 mmol, 1 eq.), copper(II) sulphate pentahydrate (0.084 mg, 0.0003 mmol, 0.1 eq.), sodium ascorbate (0.53 mg , 0.00269 mmol, 0.4 eq.) and finally with bis-amide **2.2f** (20 mg, 0.0296 mmol, 4.4 eq.) in 1 mL of THF/H_2O (1:1, THF freshly distilled and water degassed). The reaction was stirred at room temperature under nitrogen atmosphere in dark. After 18 h TLC (silica, hex:EA = 8:2 and C18, H_2O: MeOH = 1:1) indicated no presence of tetra-alkyne **3.2** and one major product. The reaction was charged to a column in order to purify by size exclusion chromatography (Sephadex LH20, MeOH). The isolated product was further purified by reverse phase flash chromatography (C18, water with gradient of MeOH from 30% to 60%) to afford 16 mg of product.



3.15

Yield: 87 %

$[\alpha]_D^{25}$ = - 4.7 (c = 0.21, MeOH)

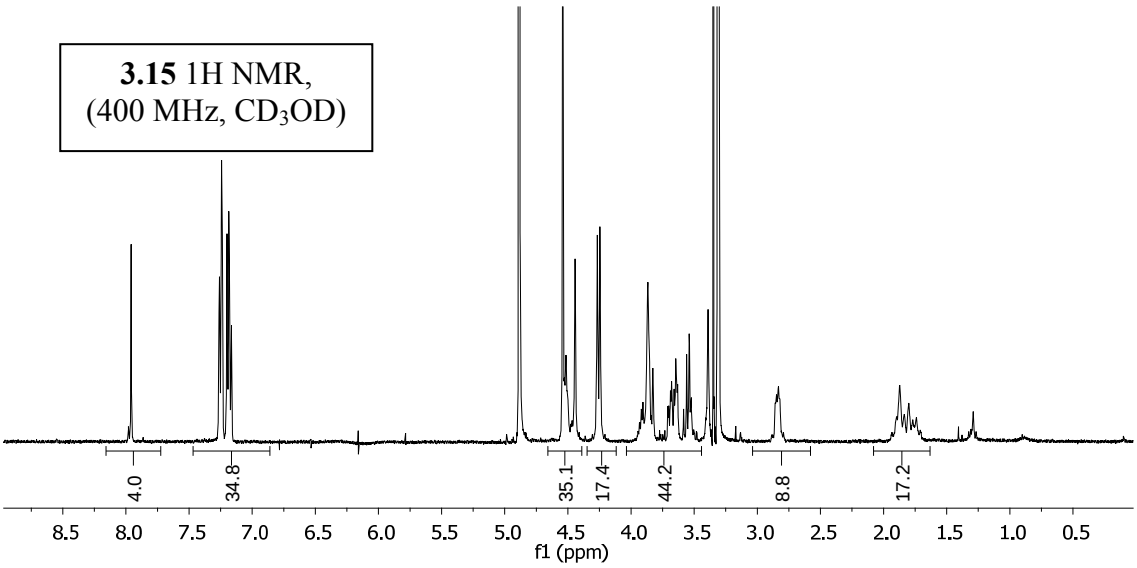
MS (MALDI, matrix: α-cyano-4-hydroxy-cinnamic acid, solvent: MeOH): calculated for $[C_{145}H_{192}N_{20}O_{48}Na]^+$: 3006.2; found = 3005.4

MS (ESI-HRMS): calculated for $[C_{145}H_{192}N_{20}O_{48}]^+$: 2981.31979; found = 2981.32444 (after deconvolution, error: 1.6 ppm)

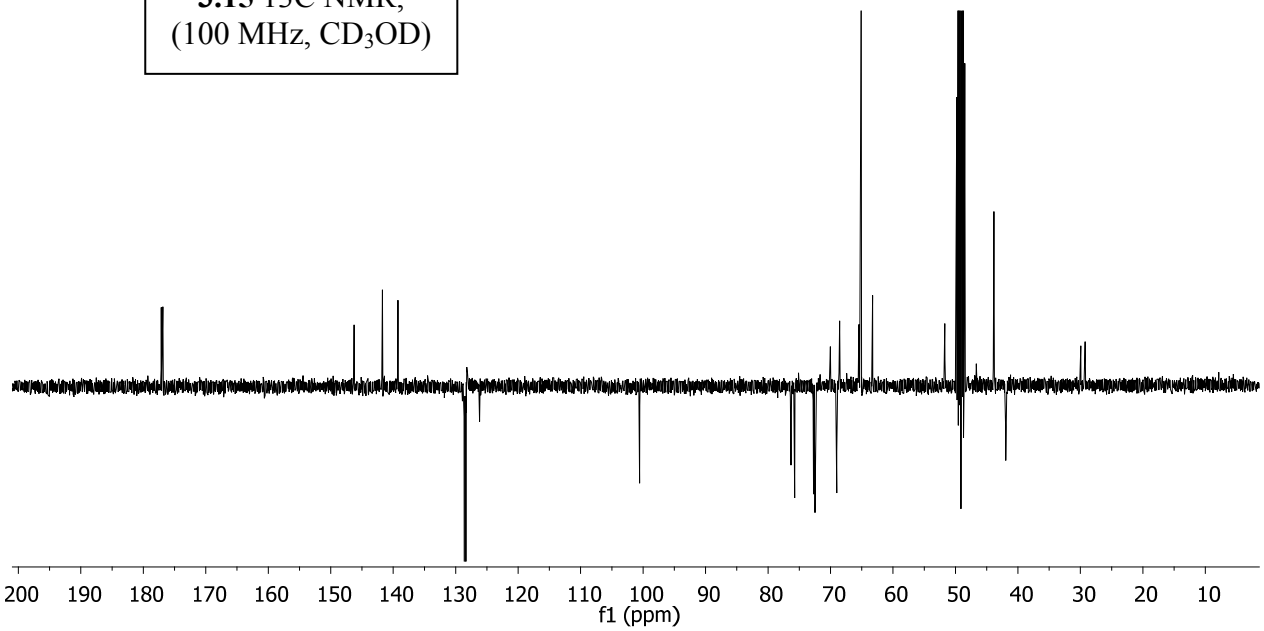
^{1}H NMR (400 MHz, CD_3OD): δ = 7.96 (s, 4H, H_{16}), 7.31 – 7.07 (m, 32H, H_{12}, H_{13}), 4.89 (br s, 4H, H_1), 4.54 (s, 16H, H_{15}), 4.51 (t, 8H, H_8, $J_{8\text{-}7}$ = 5.4 Hz), 4.44 (s, 8H, H_{18}), 4.27 (s, 8H, H_{10a}), 4.25 (s, 8H, H_{10b}), 3.95 - 3.80 (m, 20H, H_2, H_{6a}, D_2, H_7), 3.72 - 3.60 (m, 12H, H_{6b}, D_1, H_3), 3.59 - 3.49 (m, 8H, H_4, H_5), 3.44 (br s, 8H, H_{19}), 2.90 – 2.75 (m, 8H, D_4, D_5), 1.96 – 1.66 (m, 16H, D_3, D_6).

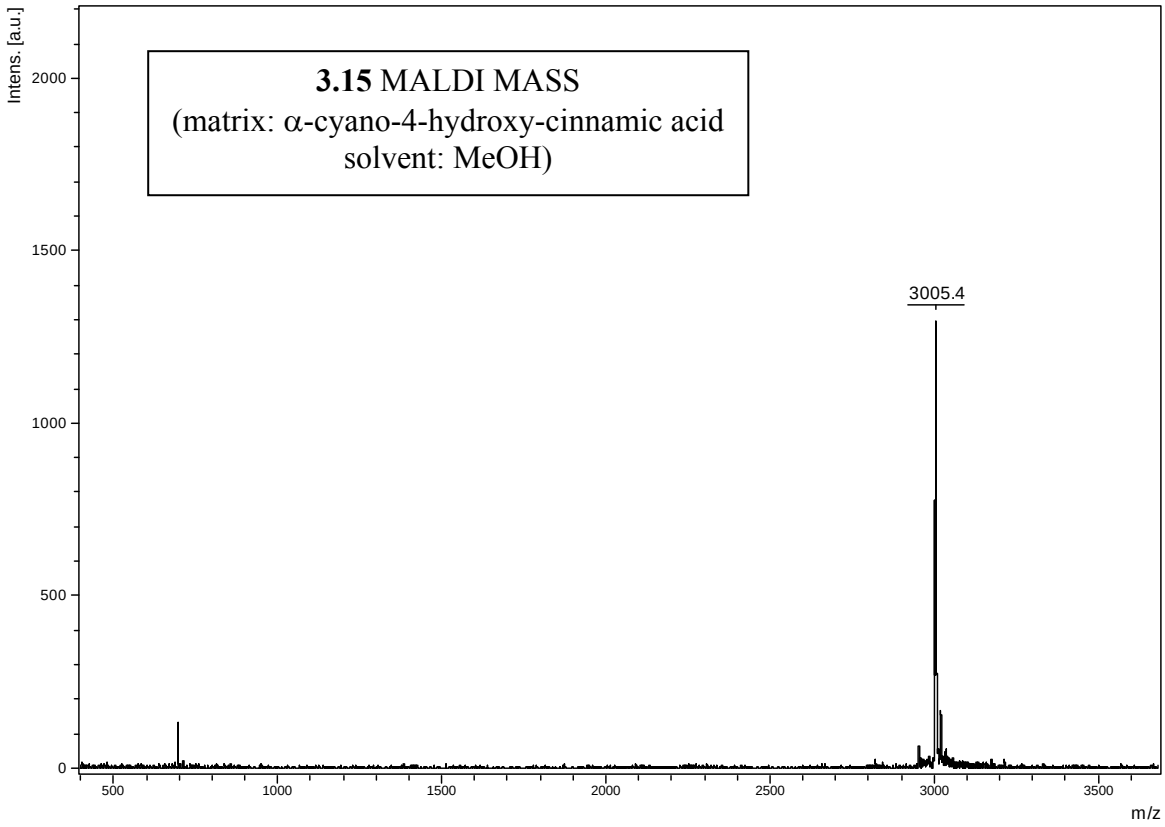
^{13}C NMR (100 MHz, CD_3OD): δ = 177.1, 176.9 (C_9); 146.3 (C_{17}); 141.7 (C_{14}); 139.2 (C_{11}); 128.5, 128.3 (C_{13}, C_{12}); 126.2 (C_{16}); 100.6 (C_1); 76.3 (C_3); 75.7 (C_{D1}); 72.7 (D_2); 72.5 (C_2); 72.4 (C_5); 70.0 (C_{19}); 69.0 (C_4); 68.5 (C_7); 65.5 (C_{18}); 65.1 (C_{15}); 63.3 (C_6); 52.6 (C_{10}); 51.7 (C_8); 46.7 (C_{20}); 43.8 (C_{10}); 41.9, 41.9 (C_{D4}, C_{D5}); 29.9, 29.2 (C_{D3}, C_{D6}).

3.15 1H NMR,
(400 MHz, CD_3OD)



3.15 13C NMR,
(100 MHz, CD_3OD)

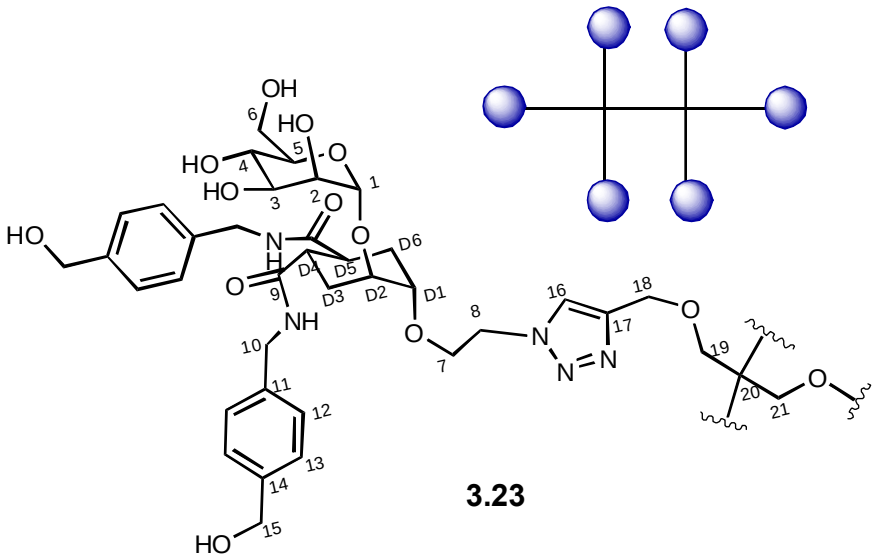




3.15 MALDI MASS
(matrix: α-cyano-4-hydroxy-cinnamic acid
solvent: MeOH)

3.5.4.8 Hexavalent glycodendrimer 3.23

A flask was charged with the following regents in the following order: hexa-alkyne **3.3** (2.04 mg, 0.00424 mmol, 1 eq.), TBTA (2.24 mg, 0.00424 mmol, 1 eq.), copper(II) sulphate pentahydrate (0.05 mg, 0.0002 mmol, 0.05 eq.), sodium ascorbate (0.33 mg , 0.0017 mmol, 0.4 eq.) and finally with bis-amide **2.2f** (20 mg, 0.0297 mmol, 7 eq.) in 1 mL of THF/H_2O (1:1, THF freshly distilled and water degassed). The reaction was stirred at room temperature under nitrogen atmosphere in dark. After 3 h TLC (silica, hex:EA = 8:2 and C18, H_2O: MeOH = 1:1) still indicated the presence of hexa-alkyne **3.3,** therefore another portion of sodium ascorbate (0.33 mg , 0.0017 mmol, 0.4 eq.) was added. The mixture was stirred overnight, then the reaction was charged to a column in order to purify by size exclusion chromatography (Sephadex LH20, MeOH). The isolated product was further purified by reverse phase flash chromatography (C18, water with gradient of MeOH from 30% to 80%) to afford 13 mg of product.



3.23

Yield: 70 %

$[\alpha]_D^{25}$ = - 2.8 (c = 0.27, MeOH)

MS (MALDI, matrix: 2,5-dihydroxybenzoic acid, solvent: MeOH)

calculated for $[C_{220}H_{292}N_{30}O_{73}]^+$: 4524.8; found = 4524.7

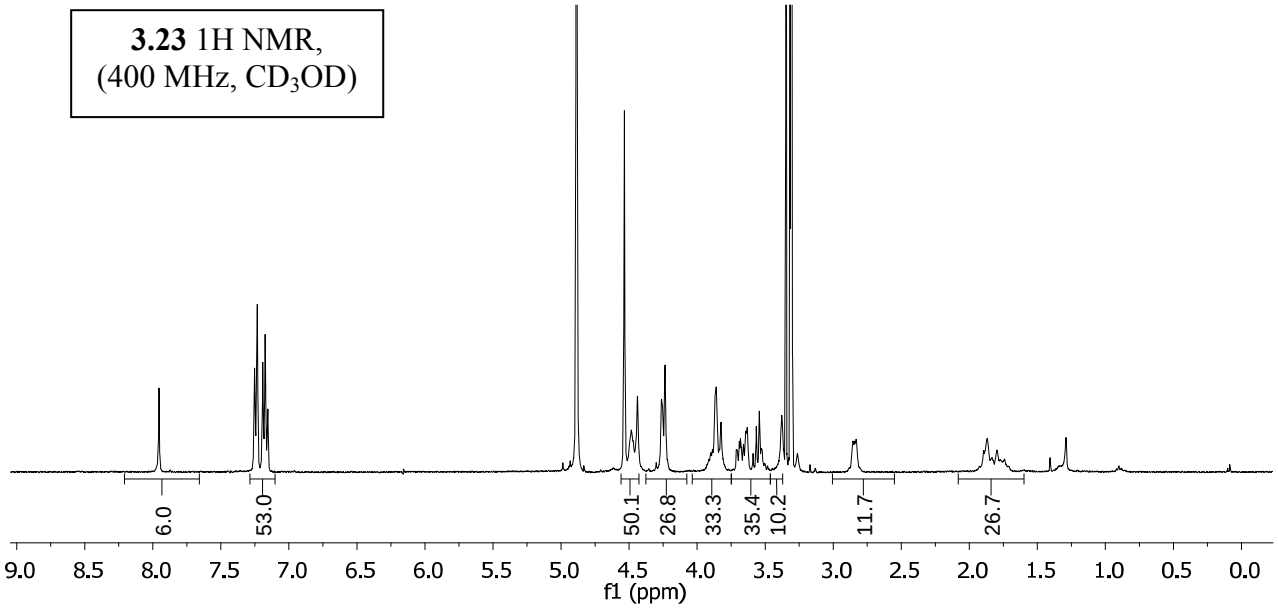
calculated for $[C_{220}H_{292}N_{30}O_{73}Na]^+$: 4547.8; found = 4549.1

MS (ESI-HRMS): calculated for $[C_{220}H_{292}N_{30}O_{73}]^+$: 4522.00590; found = 4522.01473 (after deconvolution, error: 2.0 ppm)

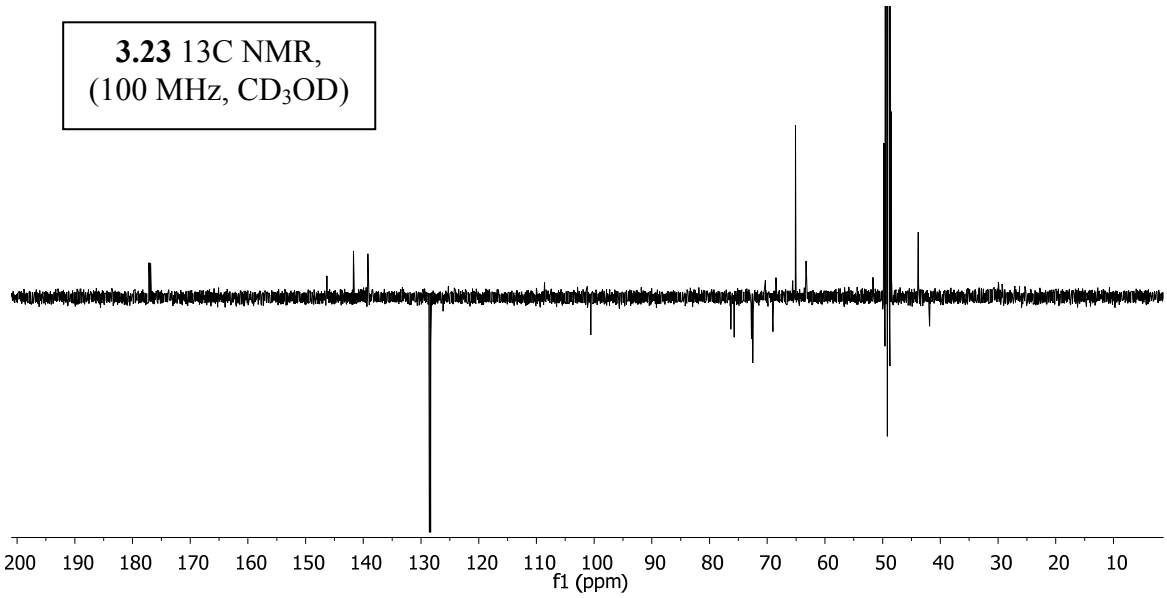
^{1}H NMR (400 MHz, CD_3OD): δ = 7.95 (s, 6H, H_{16}), 7.28 – 7.07 (m, 48H, H_{12}, H_{13}), 4.89 (br s, 6H, H_1), 4.54 (s, 24H, H_{15}), 4.51 - 4.45 (m, 12H, H_8), 4.44 (s, 12H, H_{18}), 4.28 - 4.20 (m, 24H, H_{10}), 3.95 - 3.80 (m, 30H, H_2, H_{6a}, D_2, H_7), 3.73 - 3.61 (m, 18H, H_{6b}, D_1, H_3), 3.60 - 3.47 (m, 12H, H_4, H_5), 3.38 (br s, 12H, H_{19}), 3.26 (br s, 4H, H_{21}), 2.89 – 2.78 (m, 12H, D_4, D_5), 1.93 – 1.69 (m, 24H, D_3, D_6).

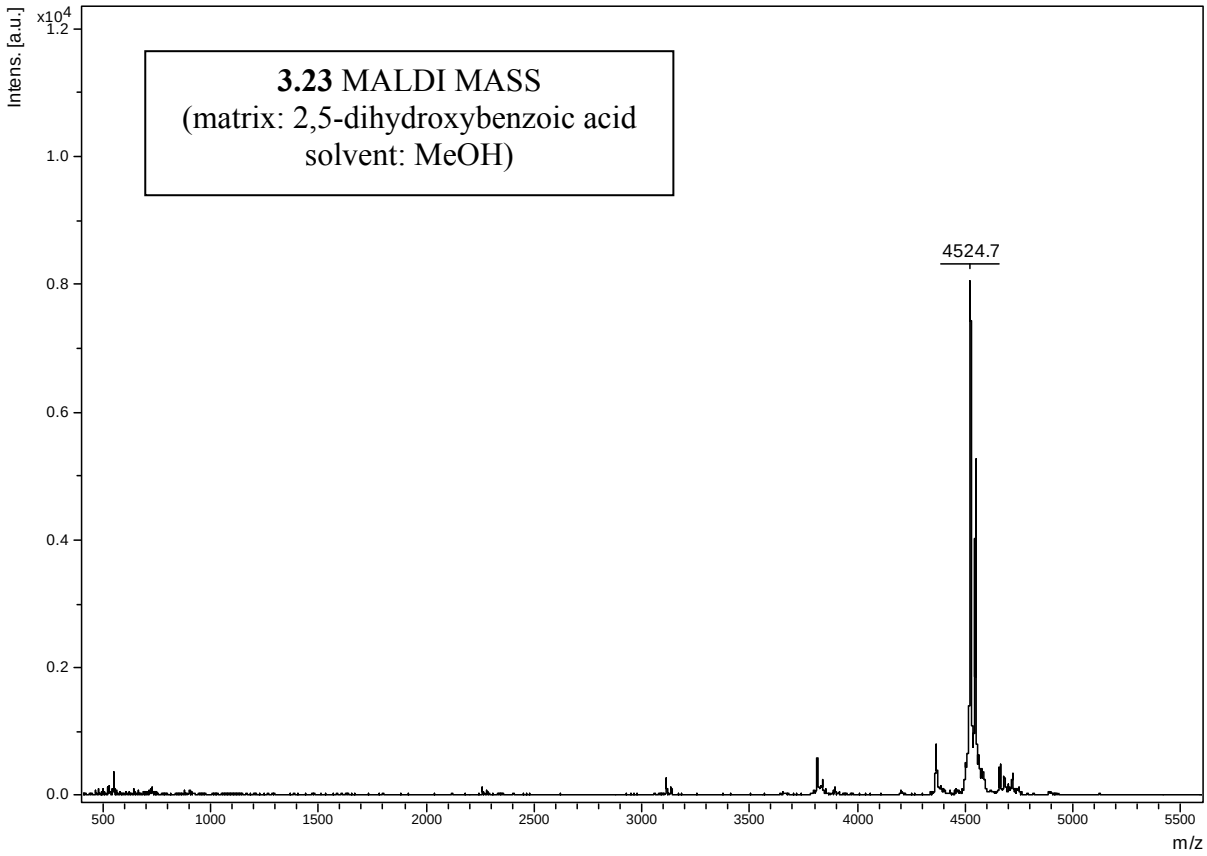
^{13}C NMR (100 MHz, CD_3OD): δ = 177.1, 176.9 (C_9); 146.3 (C_{17}); 141.7 (C_{14}); 139.2 (C_{11}); 128.6, 128.3 (C_{13}, C_{12}); 126.1 (C_{16}); 100.7 (C_1); 76.3 (C_3); 75.7 (C_{D1}); 72.7 (D_2); 72.5 (C_2, C_5); 71.1 (C_{21}); 70.3 (C_{19}); 69.0 (C_4); 68.5 (C_7); 65.5 (C_{18}); 65.1 (C_{15}); 63.3 (C_6); 52.6 (C_{10}); 51.7 (C_8); 43.8 (C_{10}); 41.9, 41.9 (C_{D4}, C_{D5}); 29.9, 29.3 (C_{D3}, C_{D6}).

3.23 1H NMR,
(400 MHz, CD_3OD)



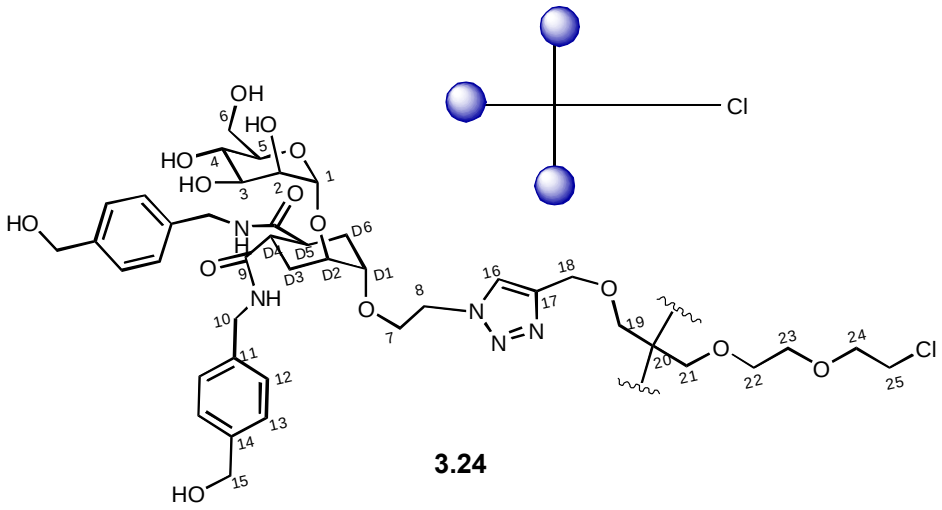
3.23 13C NMR,
(100 MHz, CD_3OD)





3.5.4.9 Trivalent glycodendron 3.24

A flask was charged with the following reagents in the following order: tris-alkyne **3.5** (8.83 mg, 0.0247 mmol, 1 eq.), TBTA (2.86 mg, 0.0054 mmol, 0.2 eq.), copper(II) sulphate pentahydrate (0.67 mg, 0.0027 mmol, 0.1 eq.), sodium ascorbate (2.13 mg , 0.0108 mmol, 0.4 eq.) and finally with bis-amide **2.2f** (60 mg, 0.0297 mmol, 3.6 eq.) in 1 mL of THF/H_2O (1:1, THF freshly distilled and water degassed). The reaction was stirred at room temperature under nitrogen atmosphere in dark. After 1 h TLC (silica, hex:EA = 8:2 and C18, H_2O: MeOH = 1:1) indicated still presence of tris-alkyne **3.5** therefore another portion of sodium ascorbate (2.13 mg , 0.0108 mmol, 0.4 eq.) was added. After an additional 2 h TLC indicated no **3.5**. The reaction was charged to a column in order to purify by size exclusion chromatography (Sephadex LH20, MeOH) to afford 47.4 mg of product.



3.24

Yield: 81 %

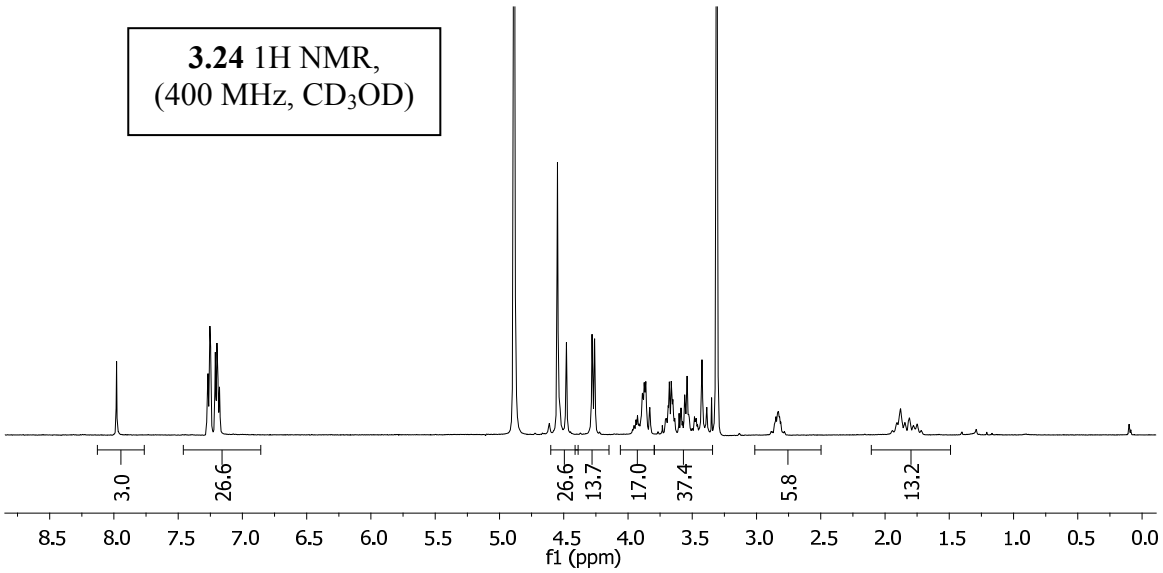
MS (MALDI matrix: sinapinic acid, solvent: MeOH):

calculated for $[C_{114}H_{154}ClN_{15}O_{38}]^+$: 2378.0; found = 2378.5

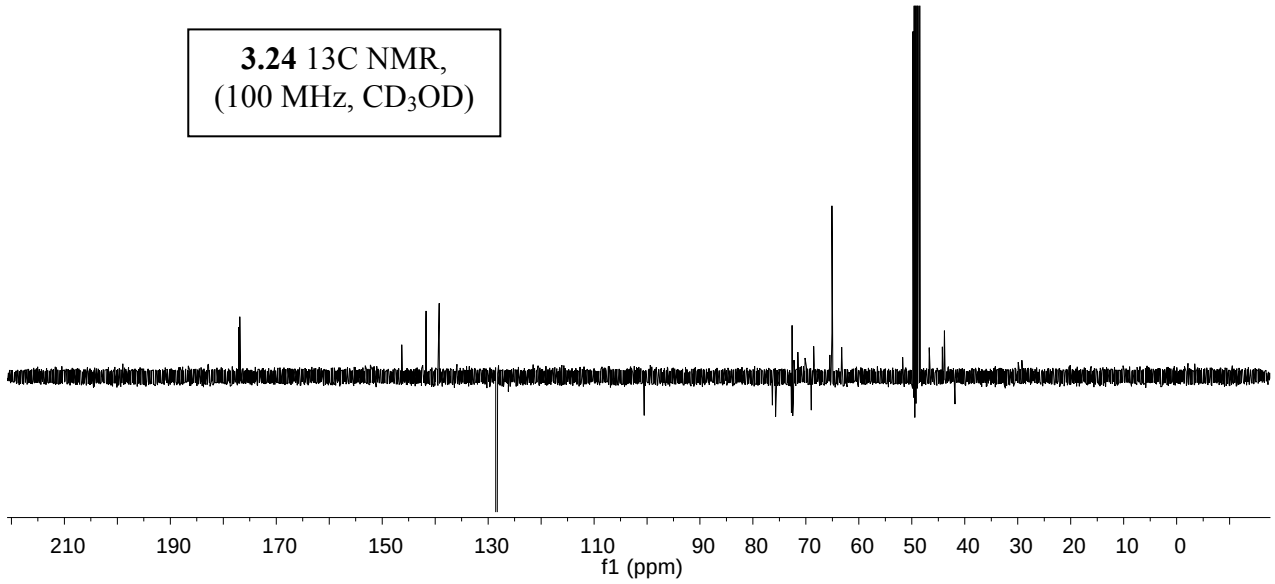
^{1}H NMR (400 MHz, CD_3OD): δ = 7.98 (s, 3H, H_{16}); 7.28 – 7.16 (m, 24H, H_{12}, H_{13}); 4.89 (br s, 3H, H_1); 4.58 – 4.50 (m, 6H, H_8); 4.55 (s, 12H, H_{15}); 4.48 (s, 6H, H_{18}); 4.28 (s, 6H, H_{10}); 4.26 (s, 6H, H_{10}); 3.99 - 3.80 (m, 15H, H_2, H_{6a}, D_2, H_7); 3.73 - 3.61 (m, 11H, H_{6b}, D_1, H_3, H_{25}); 3.61 - 3.44 (m, 12H, H_4, H_5, H_{22}, H_{23}, H_{24}), 3.42 (br s, 6H, H_{19}), 3.39 (br s, 2H, H_{21}), 2.90 – 2.76 (m, 6H, D_4, D_5), 1.96 – 1.68 (m, 12H, D_3, D_6).

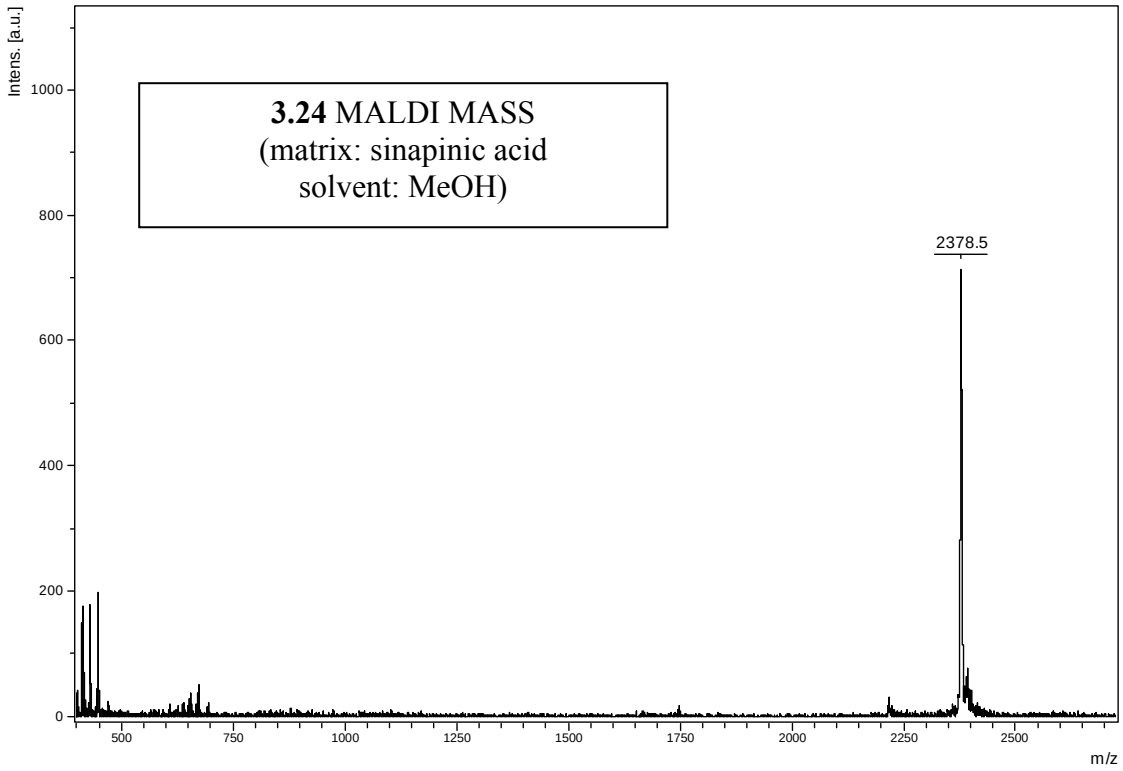
^{13}C NMR (100 MHz, CD_3OD): δ = 177.1, 176.9 (C_9), 146.3 (C_{17}), 141.7 (C_{14}), 139.2 (C_{11}), 128.5, 128.3 (C_{13}, C_{12}), 126.2 (C_{16}), 100.5 (C_1), 76.3 (C_3); 75.7 (C_{D1}); 72.7 (D_2); 72.6 (C_{24}); 72.5, 72.4 (C_2, C_5); 72.2, 71.5 (C_{22}, C_{23}); 70.9 (C_{21}); 70.2 (C_{19}); 69.0 (C_4); 68.5 (C_7); 65.5 (C_{18}); 65.1 (C_{15}); 63.3 (C_6); 51.7 (C_8); 46.7 (C_{20}); 44.2 (C_{25}); 43.8 (C_{10}); 41.9, 41.9 (C_{D4}, C_{D5}); 29.9, 29.3 (C_{D3}, C_{D6}).

3.24 1H NMR,
(400 MHz, CD_3OD)



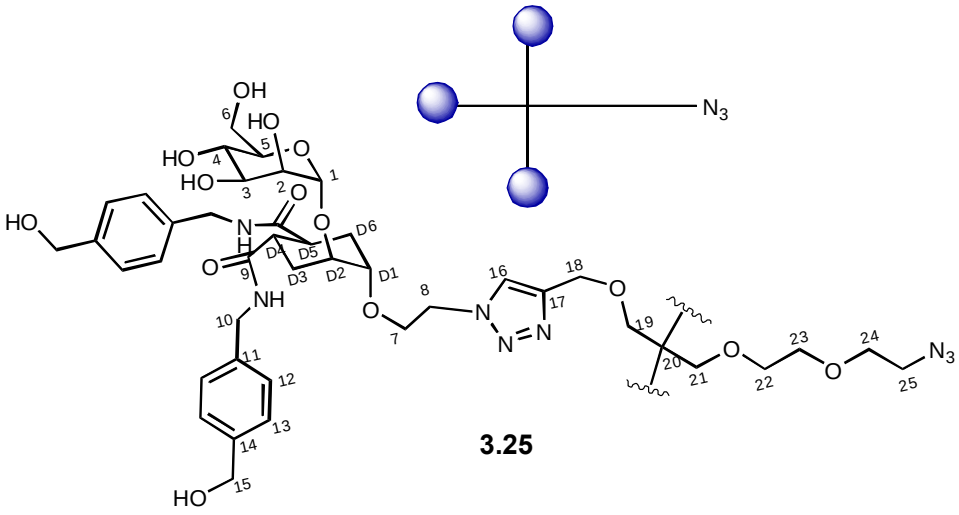
3.24 13C NMR,
(100 MHz, CD_3OD)





3.5.4.10 Trivalent glycodendron 3.25

To a solution of **3.24** (150 mg, 0.0631 mmol, 1 eq.) in DMF (1 mL) sodium azide (25 mg, 0.378 mmol, 6 eq.) was added. The reaction was stirred at 65ºC for 4 days. The solvent was removed under reduced pressure and the resulting crude was purified by reverse phase flash chromatography (C18, water with gradient of MeOH from 0% to 70%) to afford 143 mg of pure product.



3.25

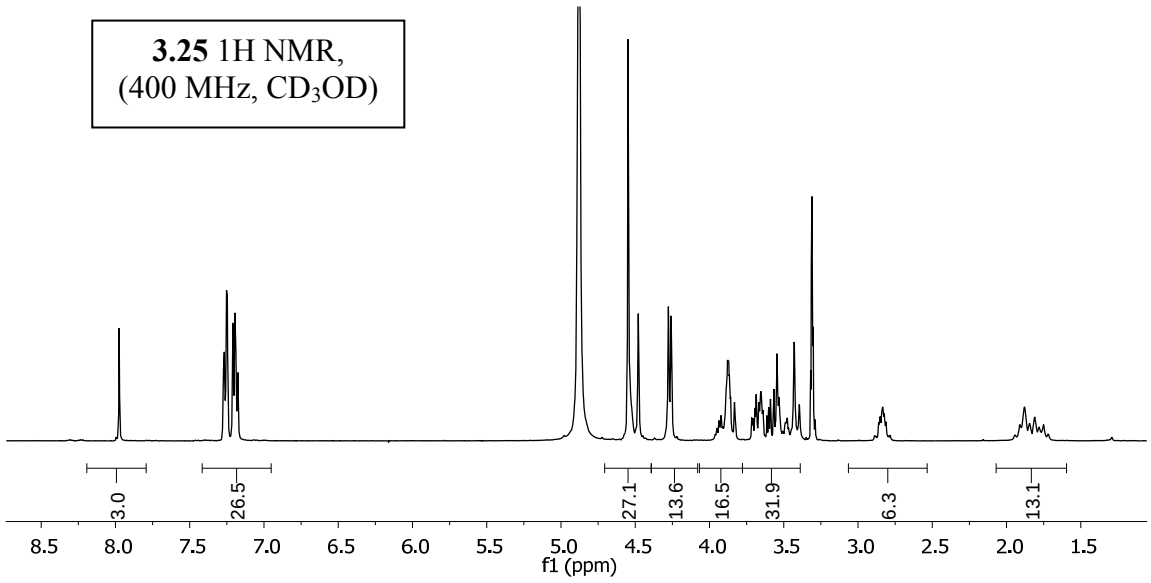
Yield: 95 %

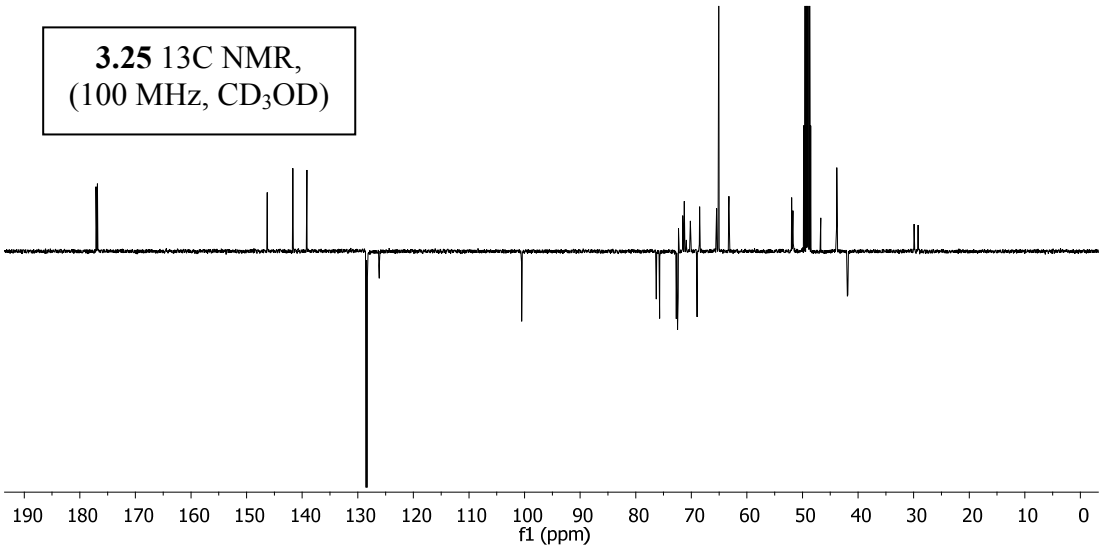
MS (MALDI matrix: sinapinic acid, solvent: MeOH):

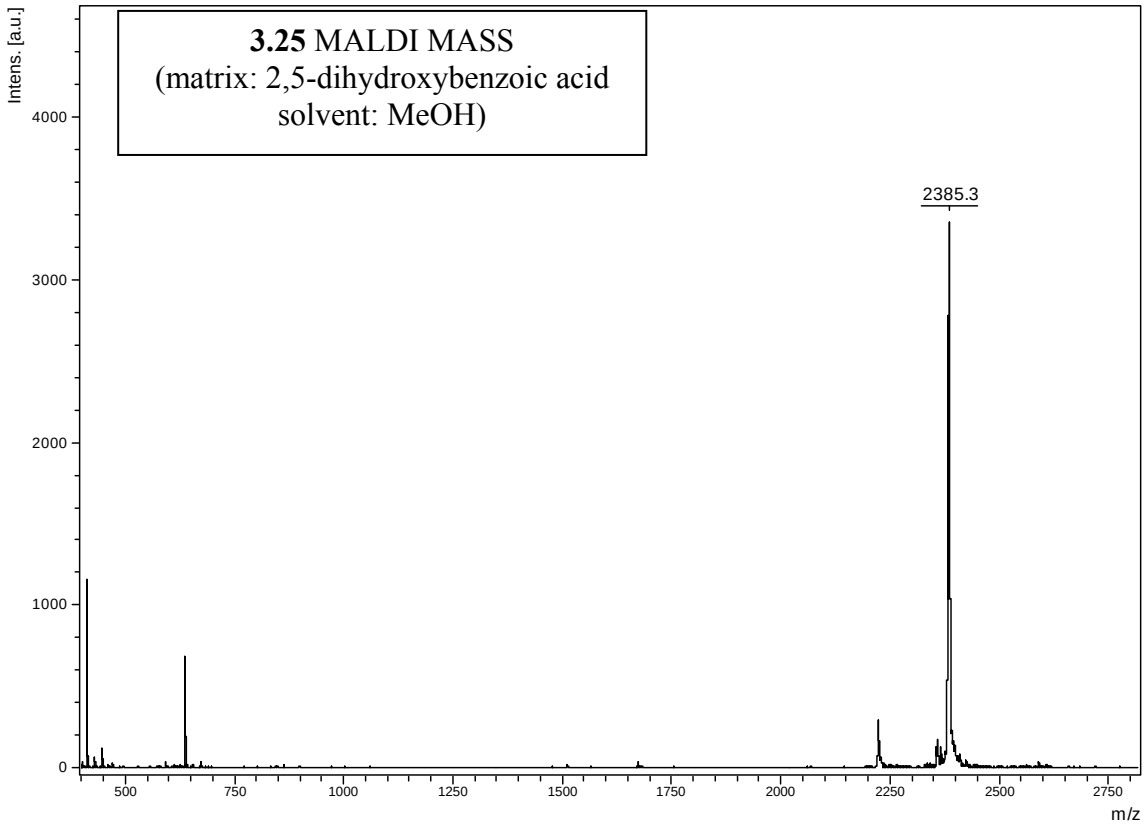
calculated for $[C_{114}H_{154}N_{18}O_{38}]^{+}$: 2384,5; found = 2385.3

^{1}H NMR (400 MHz, CD_3OD): δ = 7.98 (s, 3H, H_{16}), 7.30 – 7.13 (m, 24H, H_{12}, H_{13}), 4.89 (br s, 3H, H_1), 4.60 – 4.50 (m, 6H, H_8), 4.55 (s, 12H, H_{15}), 4.48 (s, 6H, H_{18}), 4.28 (s, 6H, H_{10}), 4.26 (s, 6H, H_{10}), 3.97 - 3.80 (m, 15H, H_2, H_{6a}, D_2, H_7), 3.73 - 3.62 (m, 9H, H_{6b}, D_1, H_3), 3.62 - 3.46 (m, 14H, H_4, H_5, H_{22}, H_{23}, H_{24}, H_{25}), 3.43 (br s, 6H, H_{19}), 3.40 (br s, 2H, H_{21}), 2.90 – 2.76 (m, 6H, D_4, D_5), 1.96 – 1.69 (m, 12H, D_3, D_6).

^{13}C NMR (100 MHz, CD_3OD): δ = 177.1, 176.8 (C_9), 146.3 (C_{17}), 141.7 (C_{14}), 139.2 (C_{11}), 128.5, 128.3 (C_{13}, C_{12}), 126.2 (C_{16}), 100.5 (C_1), 76.3 (C_3); 75.7 (C_{D1}); 72.7 (D_2); 72.5, 72.4 (C_2, C_5); 72.3, 71.5, 71.3 (C_{22}, C_{23}, C_{24}); 70.9 (C_{21}); 70.2 (C_{19}); 69.0 (C_4); 68.5 (C_7); 65.5 (C_{18}); 65.1 (C_{15}); 63.2 (C_6); 51.9 (C_{25}); 51.7 (C_8); 46.7 (C_{20}); 43.8 (C_{10}); 41.9, 41.8 (C_{D4}, C_{D5}); 29.9, 29.2 (C_{D3}, C_{D6}).



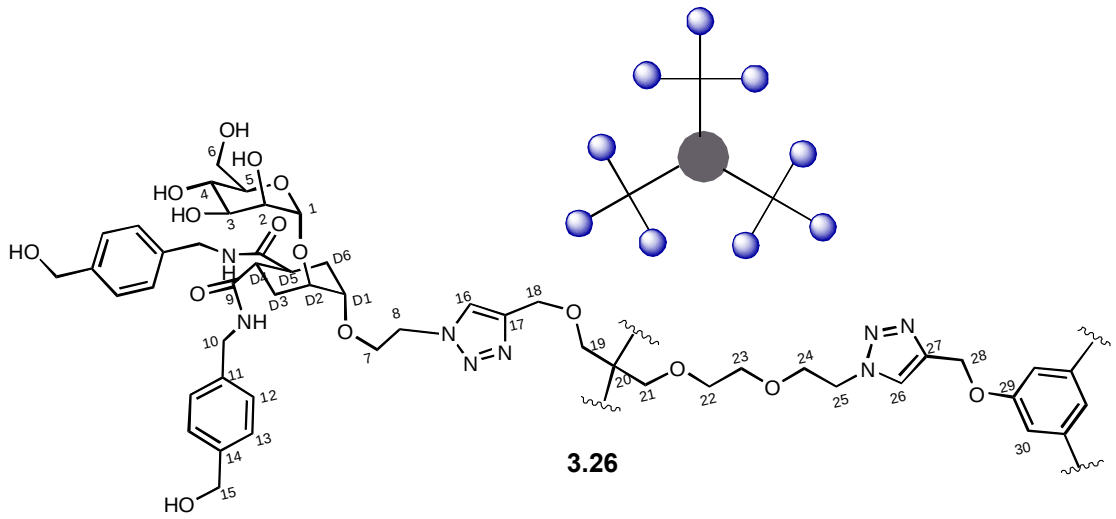




3.5.4.11 Nonavalent glycodendrimer 3.26

A flask was charged with the following reagents in the following order: tris-alkyne **3.1** (0.61 mg, 0.00838 mmol, 1 eq.), TBTA (0.8 mg, 0.0015 mmol, 0.4 eq.), copper(II) sulphate pentahydrate (0.1 mg, 0.00025 mmol, 0.1 eq.), sodium ascorbate (0.3 mg , 0.00025 mmol, 0.4 eq.) and finally with glycodendron **3.25** (20 mg, 0.00838 mmol, 3.3 eq.) in 0.8 mL of THF/H_2O (1:1, THF freshly distilled and water degassed). The reaction was stirred at room temperature under nitrogen atmosphere in dark. After 1 h TLC (silica, hex:EA = 8:2 and C18, H_2O: MeOH = 1:1) indicated still presence of tris-alkyne **3.1** and several new products (probably intermediates)

therefore another portion of sodium ascorbate (0.3 mg , 0.00025 mmol, 0.4 eq.) was added and the mixture was stirred overnight The reaction was charged to a column in order to purify by size exclusion chromatography (Sephadex LH20, MeOH) to afford 14 mg of product



3.26

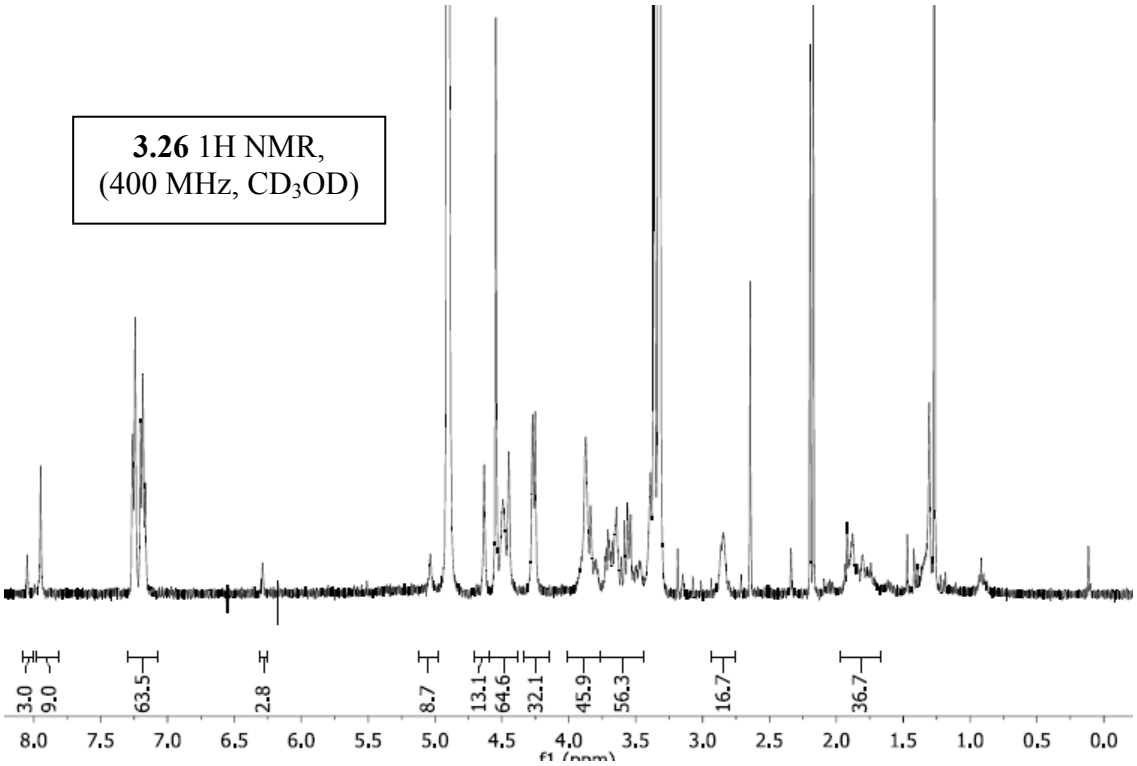
Yield: 75 %

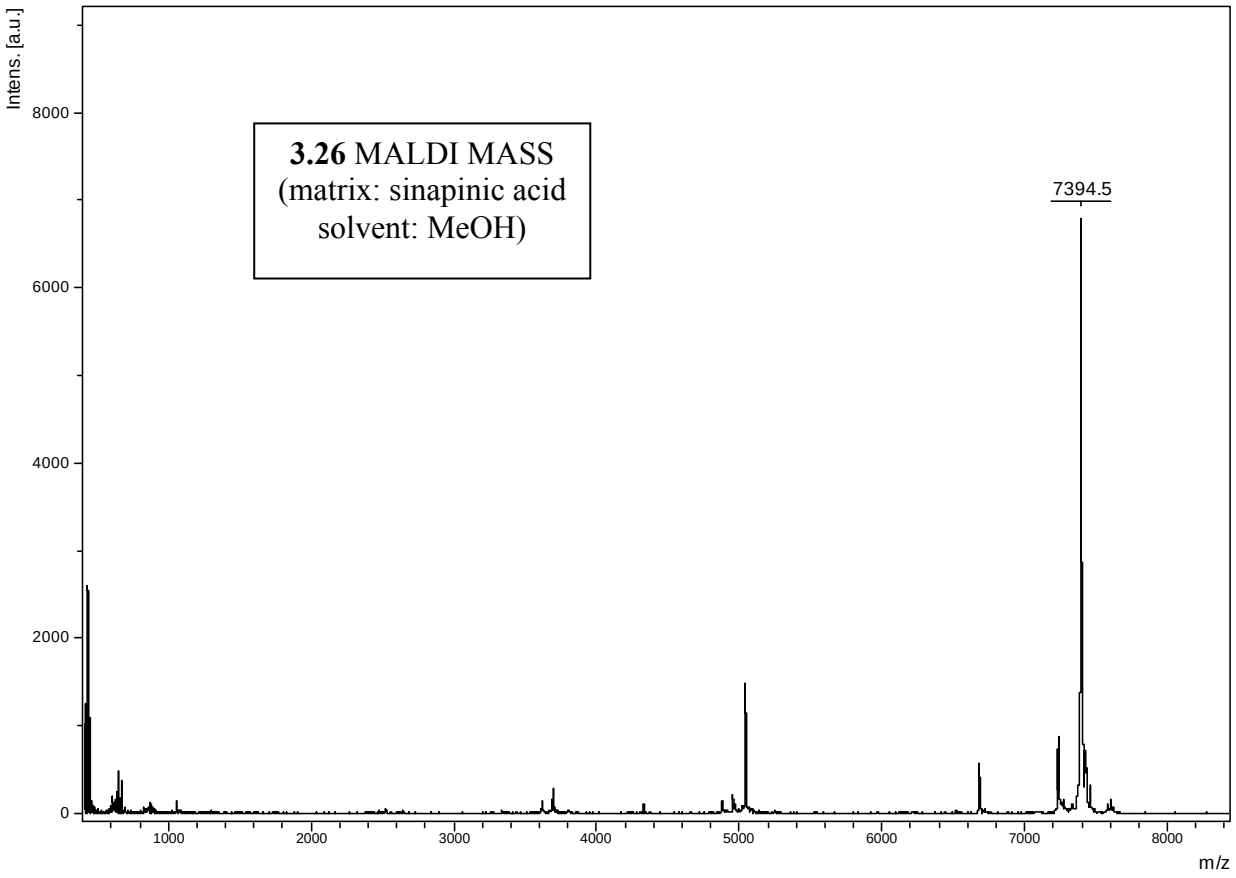
MS (MALDI, matrix: sinapinic acid, solvent: MeOH):

calculated for $[C_{357}H_{474}N_{54}O_{117}]^+$: 7393,9; found = 7394.5

MS (ESI-HRMS): calculated for $[C_{357}H_{474}N_{54}O_{117}]^+$: 7389.28007; found = 7393.28658 (after deconvolution, error: 0.7 ppm)

^{1}H NMR (400 MHz, CD_3OD): δ = 8.05 (s, 3H, H_{26}), 7.95 (s, 9H, H_{16}), 7.28 – 7.11 (m, 72H, H_{12}, H_{13}), 6.29 (br s, 3H, H_{30}), 5.03 (br s, 6H, H_{28}), 4.89 (br s, 9H, H_1), 4.63 (s, 6H, H_{25}), 4.55 (s, 36H, H_{15}), 4.52 – 4.46 (m, 18H, H_8), 4.45 (s, 12H, H_{18}), 4.27 (s, 18H, H_{10}), 4.25 (s, 18H, H_{10}), 3.96 - 3.75 (m, 51H, H_2, H_{6a}, D_2, H_7, H_{24}), 3.75 - 3.62 (m, 27H, H_{6b}, D_1, H_3), 3.61 - 3.42 (m, 30H, H_4, H_5, H_{22}, H_{23}), 3.39 (br s, 18H, H_{19}), 3.36 (br s, 6H, H_{21}), 2.89 – 2.81 (m, 18H, D_4, D_5), 1.96 – 1.68 (m, 36H, D_3, D_6).

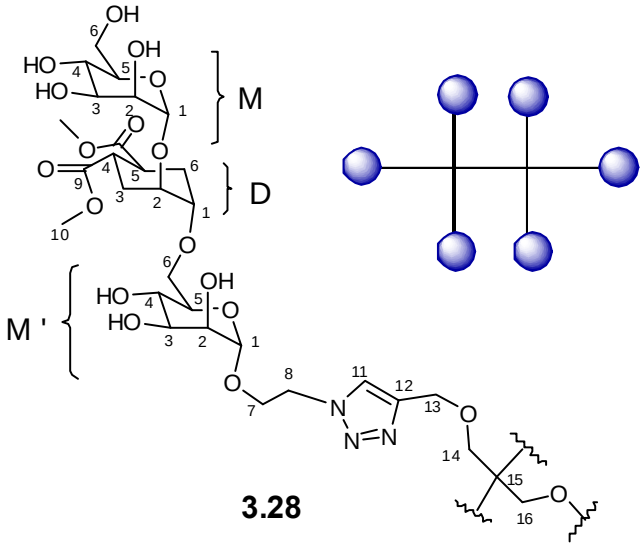




3.5.4.12 Hexavalent glycodendrimer 3.28

Pseudotrisahccaride **1.9**[17] (25 mg, 0.04 mmol, 6.6 eq.), hexavalent scaffold **3.3** (2.92 mg, 0.006 mmol, 1 eq.), copper(II) sulphate pentahydrate (0.15 mg, 0.0006 mmol, 0.1 eq.), sodium

ascorbate (0.48 mg , 0.0024 mmol, 0.4 eq.) and TBTA (0.63 mg, 0.0012 mmol, 0.2 eq.) were dissolved in 1 mL of THF/H_2O (1:1). The reaction was stirred overnight, the solvent was removed under reduced pressure and the resulting crude was purified by size exclusion chromatography (Sephadex LH20, MeOH) to afford 23 mg of product.



3.28

Yield: 90 %

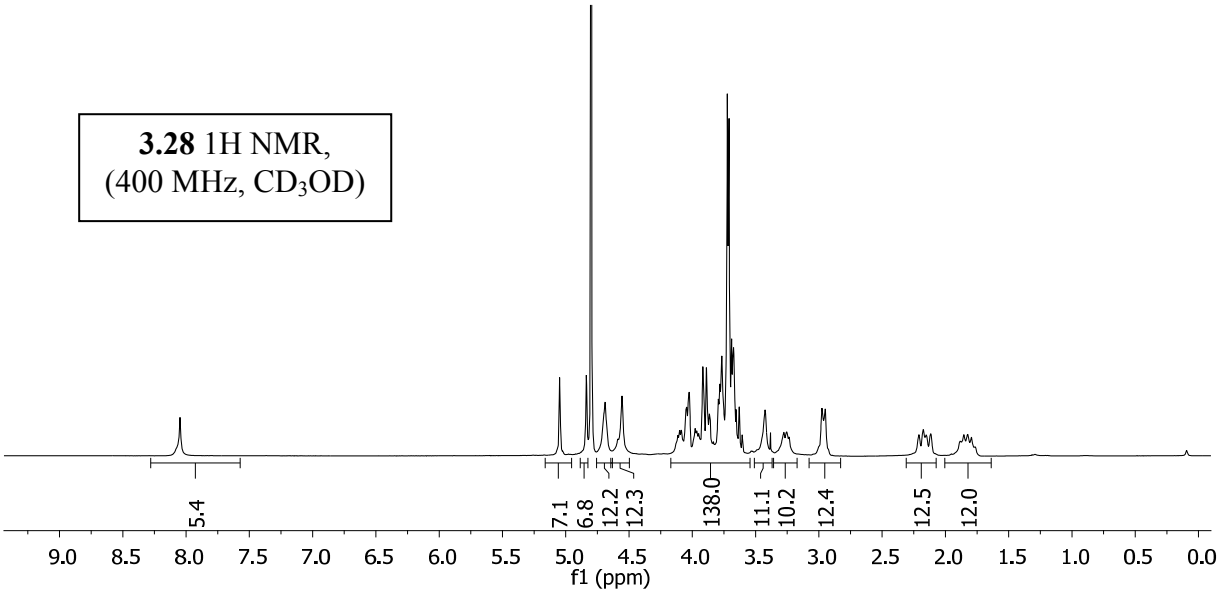
$[\alpha]_D^{25}$ = + 29.4 (c = 0.54, MeOH)

MS (ESI) calculated for $[C_{172}H_{268}N_{38}O_{206}Na]^{+}$: 4259.0; found = 4257.9

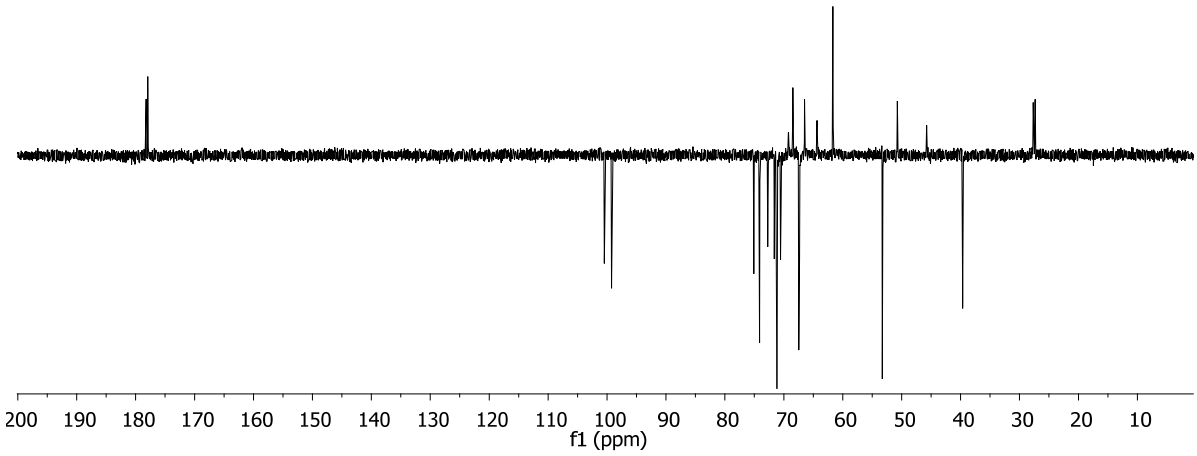
$[C_{172}H_{268}N_{38}O_{206}Na]^{+++}$: 1435.0; found = 1436.1

^{1}H NMR (400 MHz, D_2O): δ = 8.05 (s, 6H, H_{11}), 5.05 (br s, 6H, H_{1M}), 4.84 (br s, 6H, $H_{1M'}$), 4.74 – 4.63 (m, 12H, H_8), 4.55 (br s, 12H, H_{13}), 4.16 – 4.06 (m, 6H, H_{7a}), 4.06 – 3.99 (m, 12H, H_{M2}, D_2), 3.99 – 3.94 (m, 6H, H_{7b}), 3.93 - 3.81 (m, 24H, H_{6aM}, $H_{6aM'}$, $H_{2M'}$, H_{3M}), 3.82 – 3.57 (m, 84H, H_{10}, D_{1M}, H_{6bM}, $H_{6bM'}$, $H_{3M'}$, H_{4M}, $H_{4M'}$, H_{5M}, $H_{5M'}$), 3.42 (br s, 12H, H_{14}), 3.26 (br s, 4H, H_{16}), 3.03 – 2.86 (m, 12H, D_4, D_5), 2.24 – 2.05 (m, 12H, $D_{3eq.}$, $D_{6eq.}$), 1.92 - 1.67 (m, 12H, D_{3ax}, D_{6ax}).
^{13}C NMR (100 MHz, D_2O): δ = 178.2, 177.9 (C_9); 124.6 (C_{11}); 100.5 ($C_{1M'}$); 99.2 (C_{1M}); 75.1 (D_1); 74.1, 72.7 (C_{4M}, $C_{4M'}$); 71.6, 71.3, 71.2, 71.2 (C_{3M}, $C_{3M'}$, C_{2M}, $C_{2M'}$); 70.6 (D_2); 69.2 (C_{14}, C_{16}); 68.5 ($C_{6M'}$); 67.5, 67.3 (C_{5M}, $C_{5M'}$); 66.5 (C_7); 64.4 (C_{13}); 61.7 (C_{6M}); 53.3, 53.2 (C_{10}); 50.7 (C_8); 45.8 (C_{15}); 39.6 (C_4, C_5); 27.7, 27.3 (D_3, D_6).

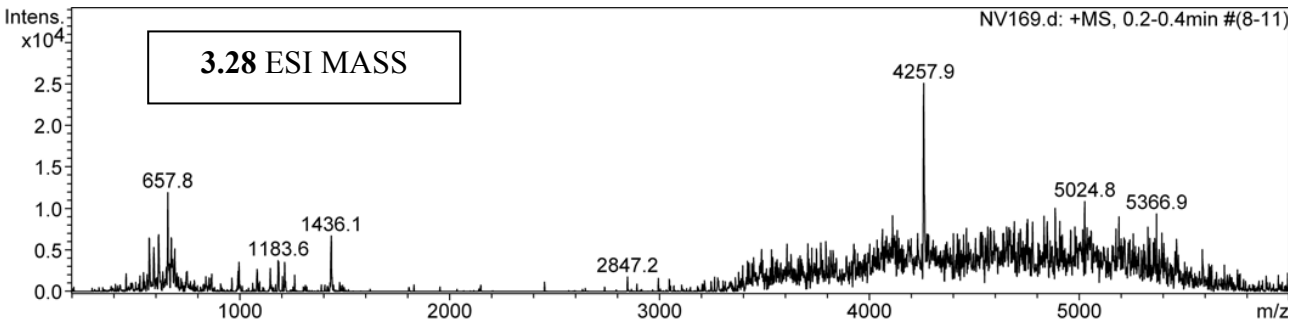
3.28 1H NMR,
(400 MHz, CD_3OD)



3.28 13C NMR,
(100 MHz, CD_3OD)



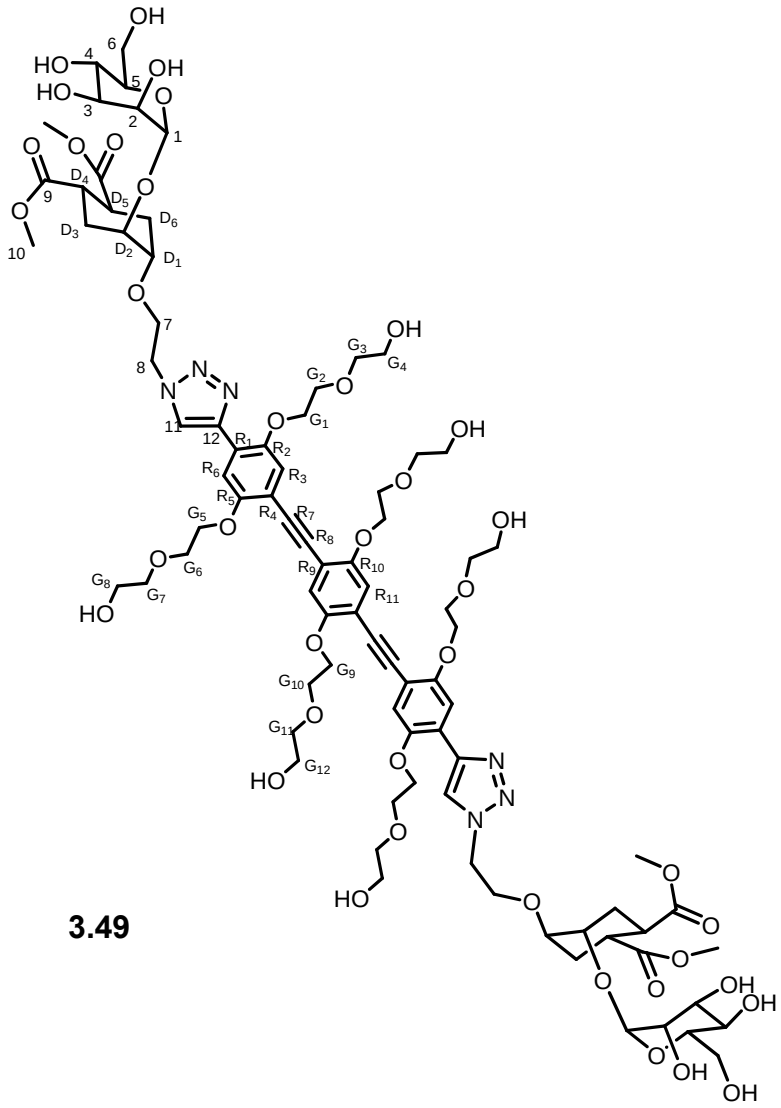
3.28 ESI MASS



3.5.5 Glycodendrimers with rods 3.32 and 3.49-3.51

3.5.5.1 Rod-like glycoconjugate 3.49

To a solution of **3.41** (5.5 mg , 0.0043 mmol, 1 eq.) in THF (0.6 mL) a 1M solution of TBAF (1 drop, cca 10 µl) was added. The reaction was stirred at room temperature. After 1 h TLC (DCM:MeOH = 8:2) indicated no starting material. Then, to the reaction mixture the following reagents were added in the following order: water (0.6 mL), TBTA (0.46 mg, 0.0008 mmol, 0.2 eq.), copper(II) sulphate pentahydrate (0.1 mg, 0.0004 mmol, 0.1 eq.), sodium ascorbate (0.34 mg , 0.0017 mmol, 0.4 eq.) and finally **1.7b** (5 mg, 0.0108 mmol, 2.5 eq.). The reaction was stirred at room temperature under nitrogen atmosphere in dark. After 2 h TLC (silica, hex:EA = 8:2 and C18, H_2O: MeOH = 1:1) indicated no **3.41**, one major and one minor product. Another portion of sodium ascorbate (0.4 eq.) was added and the mixture was stirred overnight. The reaction was charged to a column in order to purify by size exclusion chromatography (Sephadex LH20, MeOH). The isolated product was further purified by reverse phase chromatography (C18, water with gradient of methanol from 0 % to 100 %) to afford 6.2 mg of pure product.



3.49

Yield: 76 %

$[\alpha]_D^{25}$ = 16 (c = 0.27, MeOH)

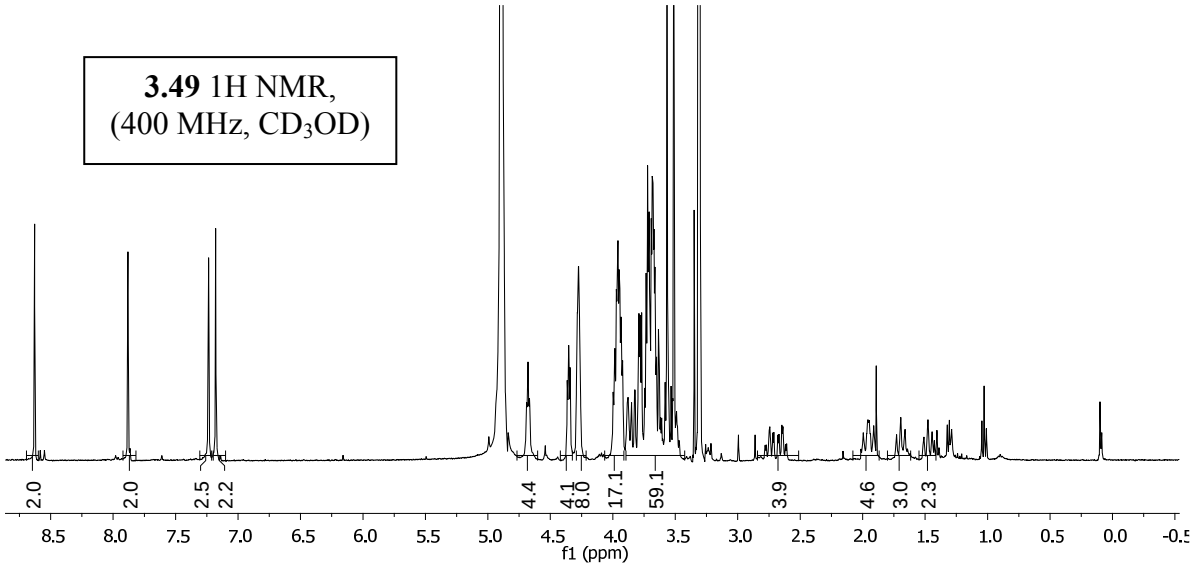
MS (HRMS) calculated for $[C_{86}H_{120}N_6O_{40}]^+$: 1876.75403; found = 1876.76014

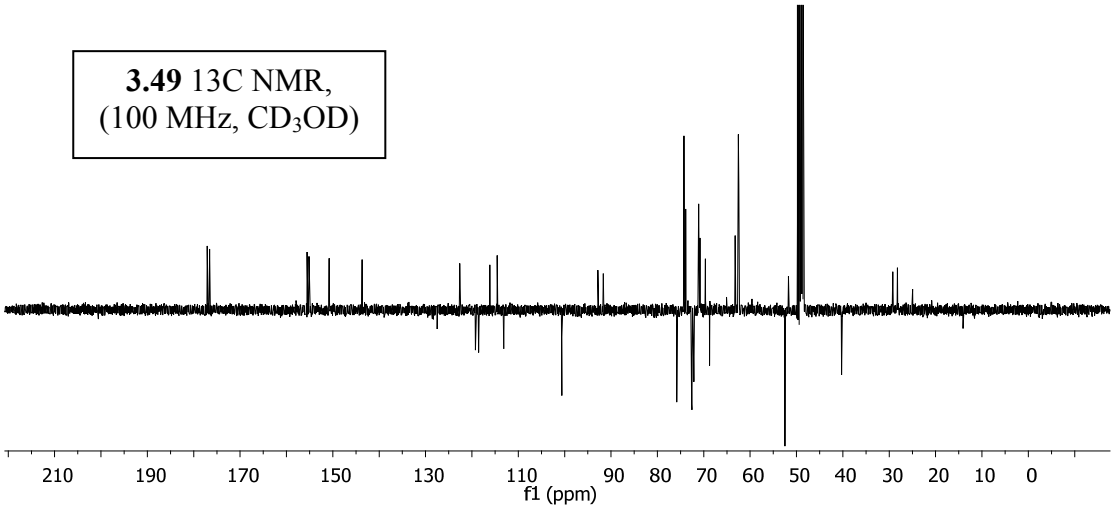
MS (ESI) calculated for $[C_{86}H_{120}N_6O_{40}]^+$: 1877.9; found = 1876.7

calculated for $[C_{86}H_{120}N_6O_{40}Na]^{++}$: 961.9; found = 961.6

^{1}H NMR (400 MHz, CD_3OD): δ = 8.63 (s, 2H, H_{11}), 7.88 (s, 2H, R_3), 7.24 (s, 2H, R_6), 7.18 (s, 2H, R_{11}), 4.89 (br s, 2H, H_1), 4.68 (t, 4H, H_8, $J_{8\text{-}7}$ = 4.7 Hz), 4.39 – 4.31 (m, 4H, G_9), 4.31 - 4.22 (m, 8H, G_1, G_5), 4.02 - 3.90 (m, 16H, G_2, G_6, G_{10}, H_7), 3.89 - 3.80 (m, 4H, H_{6a}, D_2), 3.80 - 3.75 (m, 6H, H_2, G_{12}), 3.75 - 3.59 (m, 26H, H_{6b}, D_1, H_3, G_3, G_4, G_7, G_8, G_{11}), 3.59 – 3.40 (m, 16H, H_{10}, H_5, H_4), 2.81 – 2.56 (m, 4H, D_4, D_5), 2.04 – 1.88 (m, 4H, $D_{3eq.}$, $D_{6eq.}$), 1.77 - 1.60 (m, 2H, D_{3ax} or D_{6ax}), 1.55 - 1.39 (m, 2H, D_{3ax} or D_{6ax}).

^{13}C NMR (100 MHz, CD_3OD): δ = 177.1, 176.6 (C_9); 155.5, 155.1 (R_{10}, R_5); 150.8 (R_2); 143.7 (C_{12}); 127.5 (C_{11}); 122.6 (R_1); 119.3 (R_{11}); 118.5 (R_6); 116.1, 114.6 (R_4, R_9); 113.1 (R_3); 100.6 (C_1); 92.8, 91.7 (R_7, R_8); 75.8 (C_5); 75.7 (D_1); 74.3, 74.3, 73.9 (G_3, G_7, G_{11}); 72.6, 72.6 (C_2, C_3); 72.1 (D_2); 71.2, 71.1, 71.0, 71.0, 70.8 (G_1 ,G_2, G_5, G_6, G_9, G_{10}); 69.6 (C_7); 68.7 (C_4); 63.2 (C_6); 62.5, 62.5 62.4 (G_4 ,G_8, G_{12}); 52.5 (C_{10}); 51.7 (C_8); 46.8 (C_{15}); 40.3, 40.2 (D_4, D_5); 29.2, 28.2 (D_3, D_6).

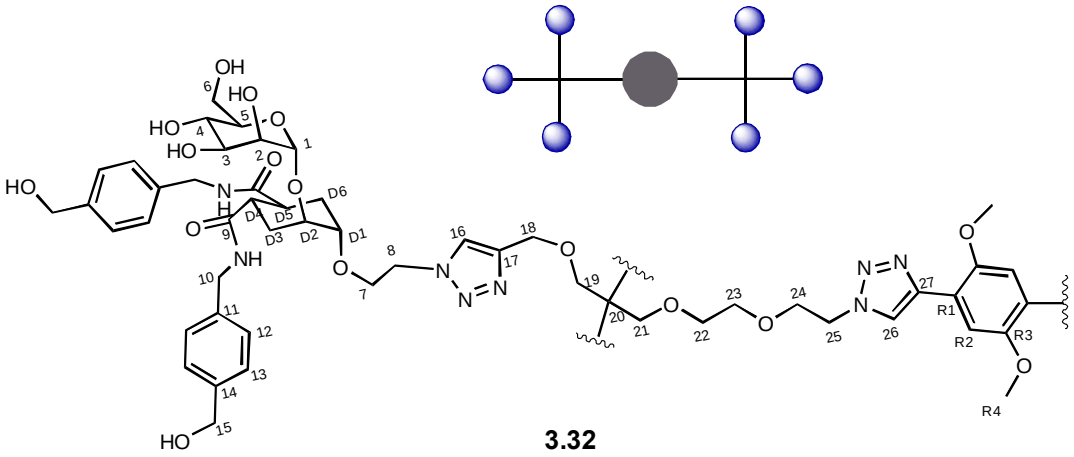




3.5.5.2 Rod-like glycodendrimer 3.32

A flask was charged with the following reagents in the following order: rod **3.7a** (0.71 mg, 0.00381 mmol, 1 eq.), TBTA (0.8 mg, 0.0015 mmol, 0.4 eq.), copper(II) sulphate pentahydrate (0.1 mg, 0.00038 mmol, 0.1 eq.), sodium ascorbate (0.3 mg , 0.00152 mmol, 0.4 eq.) and finally

with glycodendron **3.25** (20 mg, 0.00838 mmol, 2.2 eq.) in 0.8 mL of THF/H_2O (1:1, THF freshly distilled and water degassed). The reaction was stirred at room temperature under nitrogen atmosphere in dark. After 2 h TLC (silica, hex:EA = 1:1 and C18, H_2O: MeOH = 1:1) indicated still presence of rod **3.7b** and two new products (probably mono and distubstituted rod) therefore another portion of sodium ascorbate (0.4 eq.) was added and the mixture was stirred for another 1h. The reaction was charged to a column in order to purify by size exclusion chromatography (Sephadex LH20, MeOH) to afford 17 mg of product which was further purified by reverse phase chromatography (C18, water with gradient of methanol from 0 % to 80 %) to afford 8 mg of pure product.



3.32

Yield: 55 %

$[\alpha]_D^{25}$ = -9.5 (c = 0.21, MeOH)

MS (MALDI, matrix: sinapinic acid, solvent: MeOH):

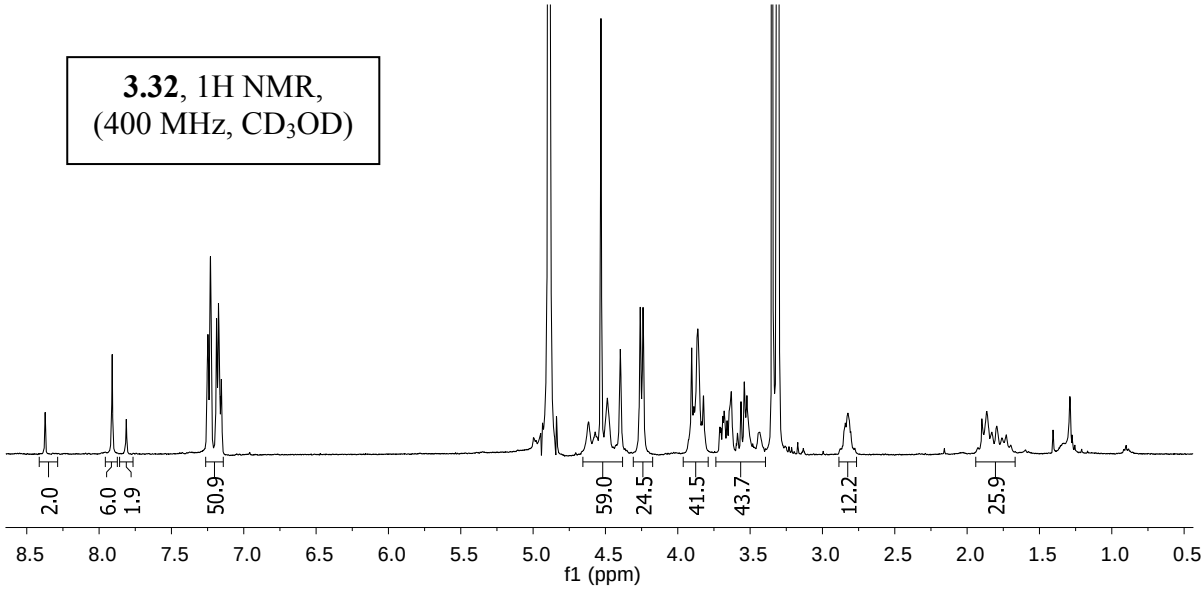
calculated for $[C_{240}H_{318}N_{36}O_{78}]^+$: 4955.3; found = 4956.0

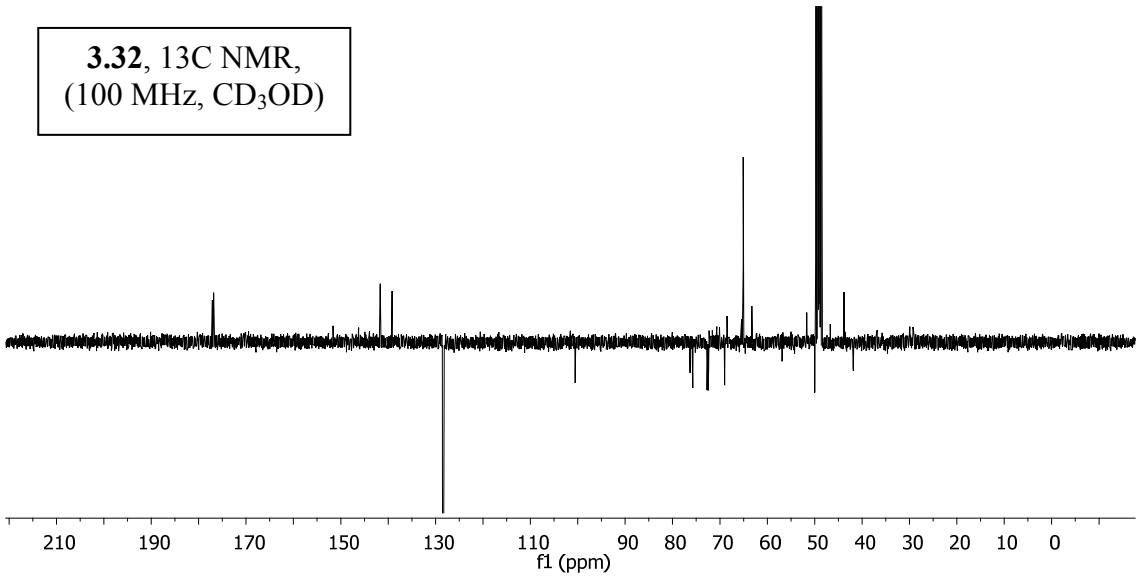
MS (ESI-HRMS): calculated for $[C_{240}H_{318}N_{36}O_{78}]^+$: 4952.20236; found = 4952.20500 (after deconvolution, error: 0.5 ppm)

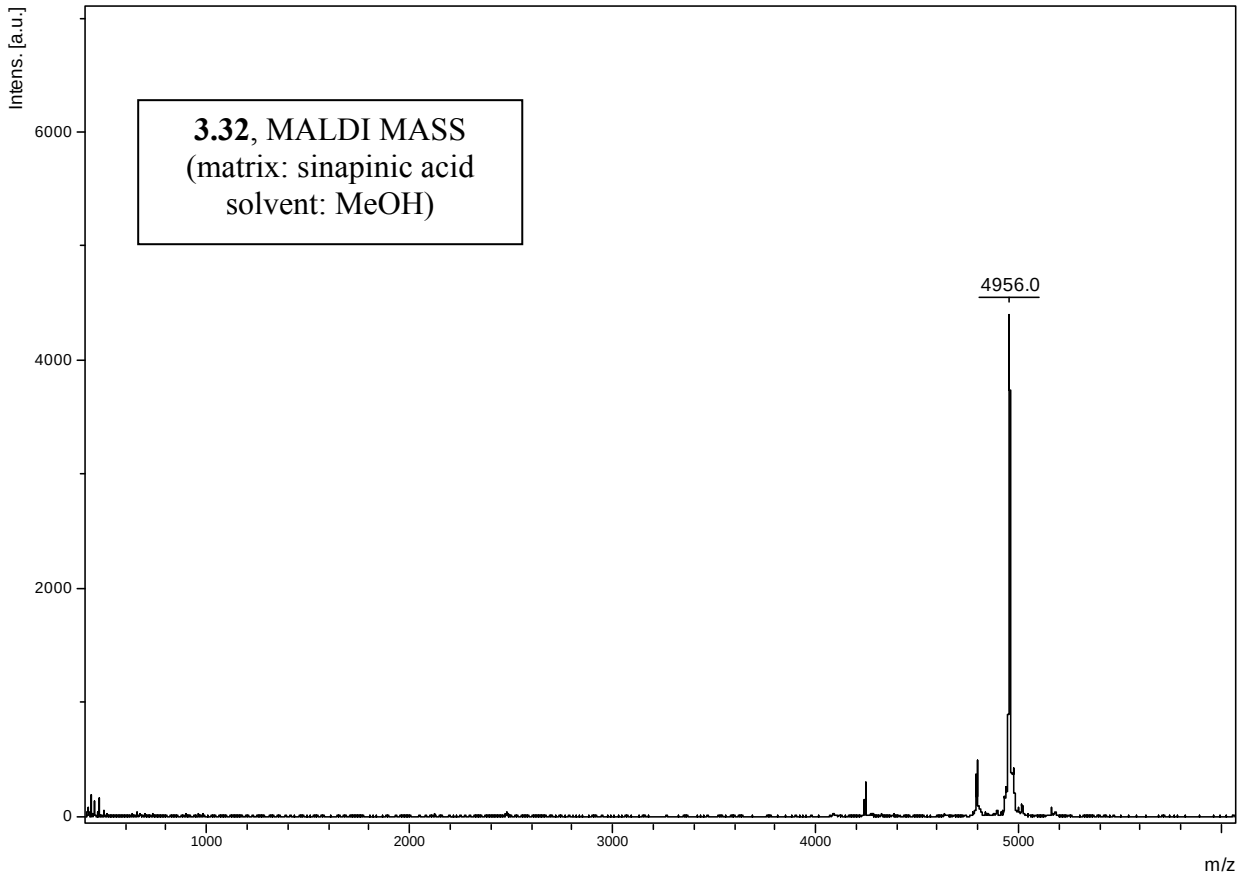
^{1}H NMR (400 MHz, CD_3OD): δ = 8.37 (s, 2H, H_{26}), 7.91 (s, 6H, H_{16}), 7.81 (s, 2H, R_2),7.27 – 7.11 (m, 48H, H_{12}, H_{13}), 4.89 (br s, 6H, H_1), 4.59 – 4.54 (m, 4H, H_{25}), 4.53 (s, 24H, H_{15}), 4.51 – 4.45 (m, 12H, H_8), 4.40 (br s, 12H, H_{18}), 4.26 (s, 12H, H_{10a}), 4.24 (s, 12H, H_{10b}), 3.95 - 3.79 (m, 40H, H_2, H_{6a}, D_2, H_7, H_{24}, R_4), 3.72 - 3.57 (m, 18H, H_{6b}, D_1, H_3), 3.56 - 3.40 (m, 20H, H_4, H_5,

H_{22}, H_{23}), 3.38 – 3.27 (m, 16H, H_{19}, H_{21}), 2.89 – 2.75 (m, 12H, D_4, D_5), 1.96 – 1.67 (m, 24H, D_3, D_6).

^{13}C NMR (100 MHz, CD_3OD): δ = 177.1, 176.8 (C_9); 151.6 (R_3); 146.3 (C_{17}); 144.9 (C_{27}); 141.7, 141.7 (C_{14}); 139.2, 139.2 (C_{11}); 128.5, 128.5 128.3 (C_{13}, C_{12}); 126.4 (C_{26}); 126.0 (C_{16}); 111.1 (R_2); 100.5 (C_1); 76.3 (C_3); 75.7 (C_{D1}); 72.7 (D_2); 72.5, 72.4 (C_2, C_5); 72.3, 71.6 (C_{22}, C_{23}); 70.6, 70.1 (C_{21}, C_{19}); 70.2 (C_{19}); 69.0 (C_4); 68.5 (C_7); 65.4 (C_{18}); 65.1 (C_{15}); 63.2 (C_6); 56.9 (R_4); 51.7 (C_{25}, C_8); 46.7 (C_{20}); 43.8 (C_{10}); 41.9, 41.8 (C_{D4}, C_{D5}); 29.9, 29.2 (C_{D3}, C_{D6}).

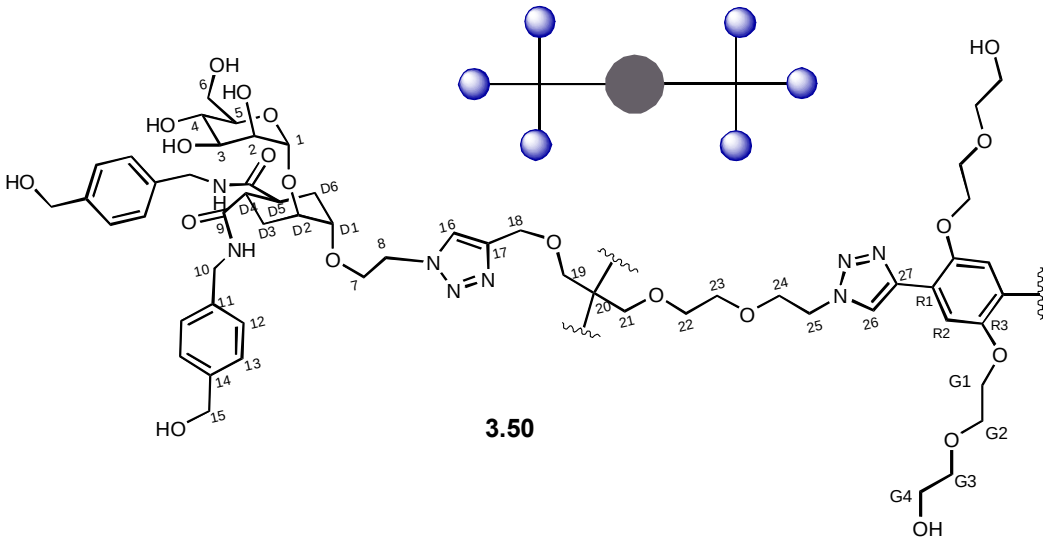






3.5.5.3 Rod-like glycodendrimer 3.50

A flask was charged with the following reagents in the following order: rod **3.7b** (1.27 mg, 0.00381 mmol, 1 eq.), TBTA (0.8 mg, 0.0015 mmol, 0.4 eq.), copper(II) sulphate pentahydrate (0.1 mg, 0.00038 mmol, 0.1 eq.), sodium ascorbate (0.3 mg , 0.00152 mmol, 0.4 eq.) and finally with glycodendron **3.25** (20 mg, 0.00838 mmol, 2.2 eq.) in 0.8 mL of THF/H_2O (1:1, THF freshly distilled and water degassed). The reaction was stirred at room temperature under nitrogen atmosphere in dark. After 4 h TLC (silica, hex:EA = 1:1 and C18, H_2O: MeOH = 1:1) indicated still presence of rod **3.7b** and of two new products (probably mono and distubstituted rod) therefore another portion of sodium ascorbate (0.4 eq.) was added and the mixture was stirred for another 1h. The reaction was charged to a column in order to purify the product by size exclusion chromatography (Sephadex LH20, MeOH). The isolated product was further purified by reverse phase chromatography (C18, water with gradient of methanol from 0 % to 80 %) to afford 9.1 mg of pure product.



3.50

Yield: 45%

$[\alpha]_D^{25}$ = -18.9 (c =0.1, MeOH)

MS (MALDI, matrix: sinapinic acid, solvent: MeOH):

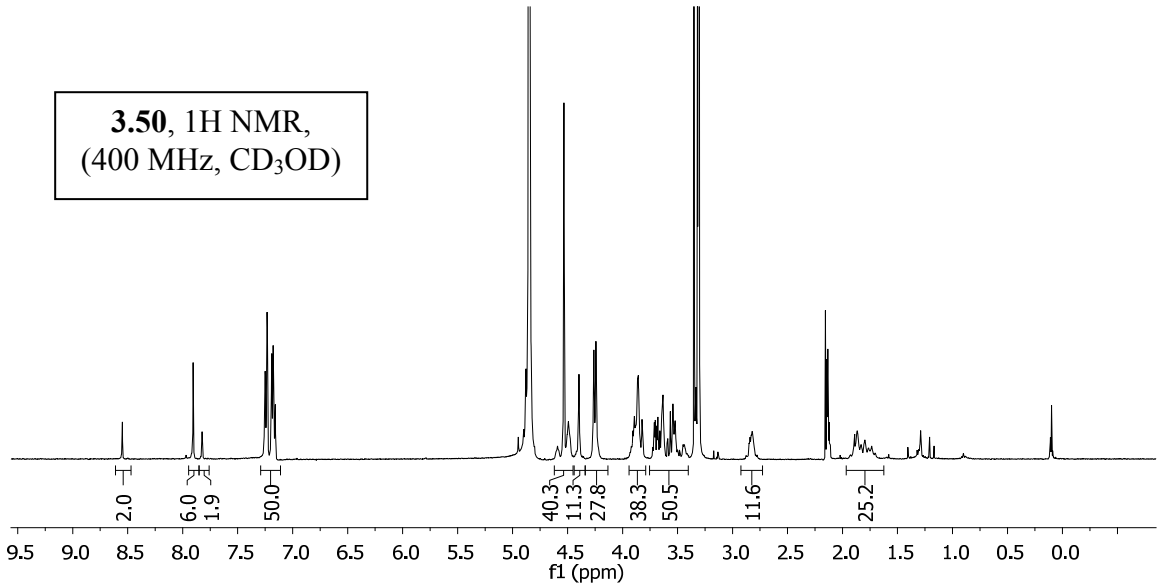
calculated for $[C_{246}H_{330}N_{36}O_{82}]^+$: 5103.4; found = 5102.6

MS (ESI-HRMS): calculated for $[C_{246}H_{330}N_{36}O_{82}]^+$: 5100.27592; found = 5100.29384 (after deconvolution, error: 3.5 ppm)

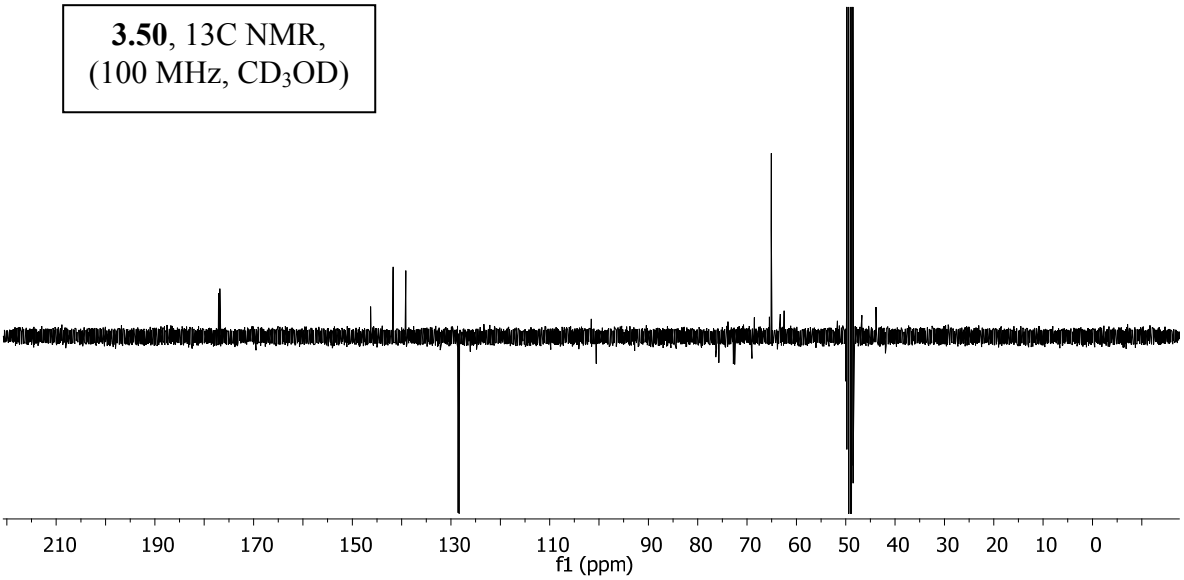
^{1}H NMR (400 MHz, CD_3OD): δ = 8.55 (s, 2H, H_{26}), 7.90 (s, 6H, H_{16}), 7.82 (s, 2H, R_2), 7.27 – 7.12 (m, 48H, H_{12}, H_{13}), 4.88 (d, 6H, H_1, $J_{1\text{-}2}$ = 1.4 Hz), 4.61 – 4.56 (m, 4H, H_{25}), 4.53 (s, 24H, H_{15}), 4.50 – 4.44 (m, 12H, H_8), 4.40 (s, 12H, H_{18}), 4.26 (s, 12H, H_{10a}), 4.24 (s, 12H, H_{10b}), 3.94 - 3.80 (m, 38H, H_2, H_{6a}, D_2, H_7, H_{24}, G_1), 3.74 - 3.61 (m, 26H, H_{6b}, D_1, H_3, G_3, G_2), 3.60 - 3.38 (m, 24H, H_4, H_5, H_{22}, H_{23}, G_4), 3.33 (s, 12H, H_{19}), 3.33 – 3.27 (s, 4H, H_{21}), 2.91 – 2.74 (m, 12H, D_4, D_5), 1.96 – 1.66 (m, 24H, D_3, D_6).

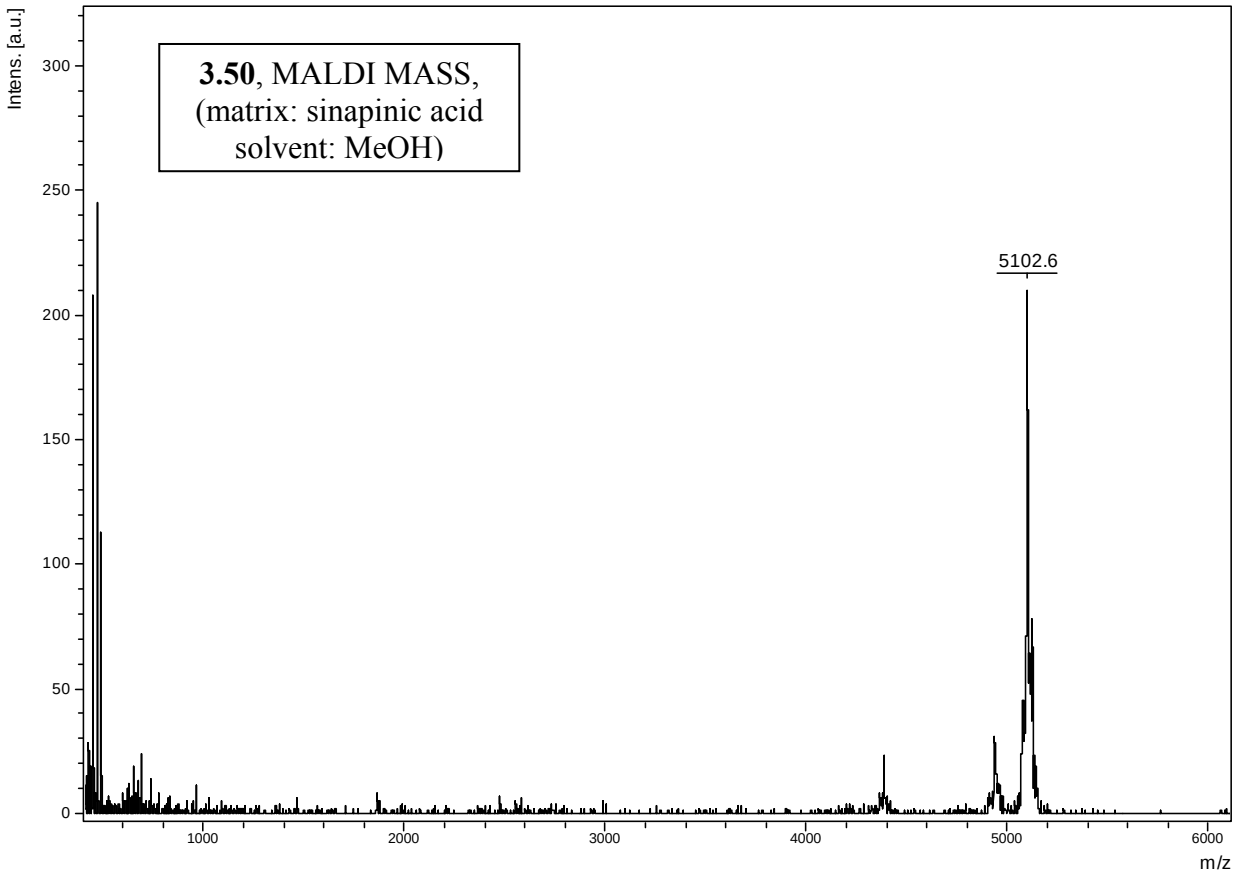
^{13}C NMR (100 MHz, CD_3OD): δ = 177.1, 176.8 (C_9); 146.3 (C_{17}); 141.7, 141.7 (C_{14}); 139.2, 139.2 (C_{11}); 128.6, 128.5, 128.3, 128.3 (C_{13}, C_{12}); 126.4 (C_{26}); 126.1 (C_{16}); 112.6 (R_2); 100.6 (C_1); 76.3 (C_3); 75.7 (C_{D1}); 73.9 (G_3); 72.7 (D_2); 72.5, 72.4 (C_2, C_5); 72.3 – 69.0 (C_{22}, C_{23}, C_{21}, C_{19}, G_1); 69.0 (C_4); 68.5 (C_7); 65.5 (C_{18}); 65.1 (C_{15}); 63.3 (C_6); 62.5 (G_2); 51.7 (C_{25}, C_8); 46.7 (C_{20}); 43.9 (C_{10}); 41.9 (C_{D4}, C_{D5}); 29.7, 29.1 (C_{D3}, C_{D6}).

3.50, 1H NMR,
(400 MHz, CD_3OD)



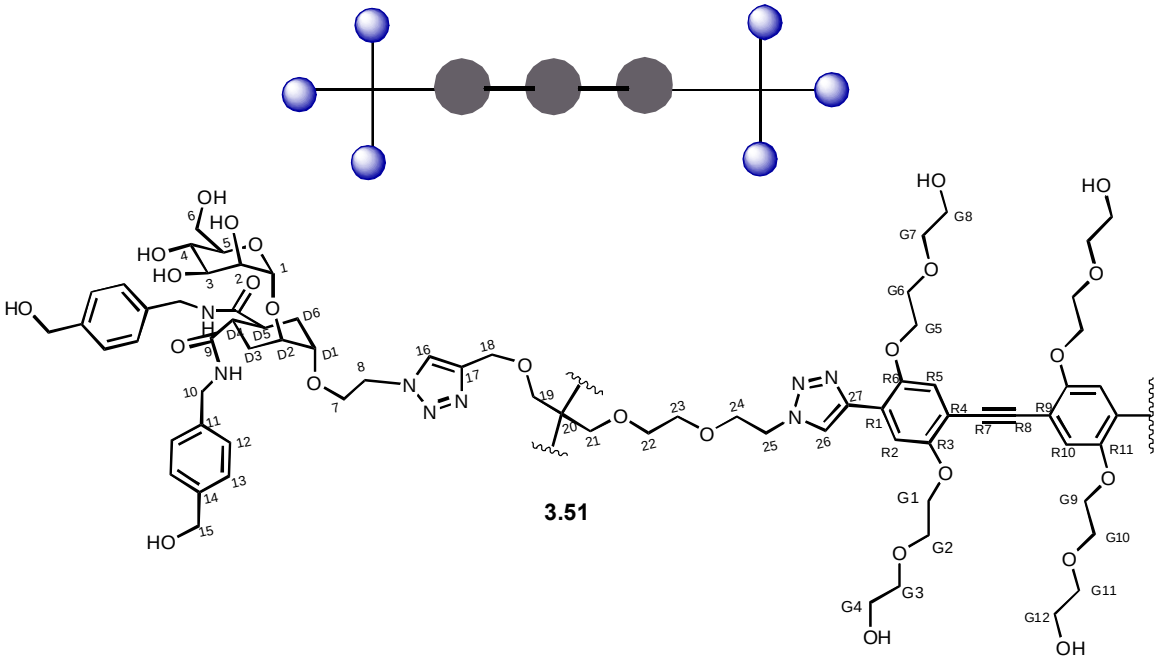
3.50, 13C NMR,
(100 MHz, CD_3OD)





3.5.5.4 Rod-like glycodendrimer 3.51

To a solution of **3.41** (4.8 mg , 0.00379 mmol, 1 eq.) in THF (0.4 mL) a 1M solution of TBAF (1 drop, ca 10 µl) was added. The reaction was stirred at room temperature. After 3 h TLC (DCM:MeOH = 8.5:1.5) indicated no starting material. Then, to the reaction mixture the following reagents were added in the following order: water (0.6 mL), TBTA (0.8 mg, 0.0015 mmol, 0.4 eq.), copper(II) sulphate pentahydrate (0.2 mg, 0.00038 mmol, 0.2 eq.), sodium ascorbate (0.3 mg , 0.00152 mmol, 0.4 eq.) and finally glycodendron **3.27** (20 mg, 0.00873 mmol, 2.3 eq.). The reaction was stirred at room temperature under nitrogen atmosphere in dark. After 2 h TLC (silica, hex:EA:H2O = 7:3:0.3) indicated no **3.41**, one major and one minor product. Another portion of sodium ascorbate (0.4 eq.) was added and the mixture was stirred overnight The reaction was charged to a column in order to purify the product by size exclusion chromatography (Sephadex LH20, MeOH) to afford 12.5 mg of product.



yield: 58%

$[\alpha]_D^{25}$ = -8.1 (c = 0.22, MeOH)

MS (MALDI, matrix: sinapinic acid, solvent: MeOH):

calculated for $[C_{278}H_{370}N_{36}O_{94}Na]^+$: 5743.1; found = 5744.6

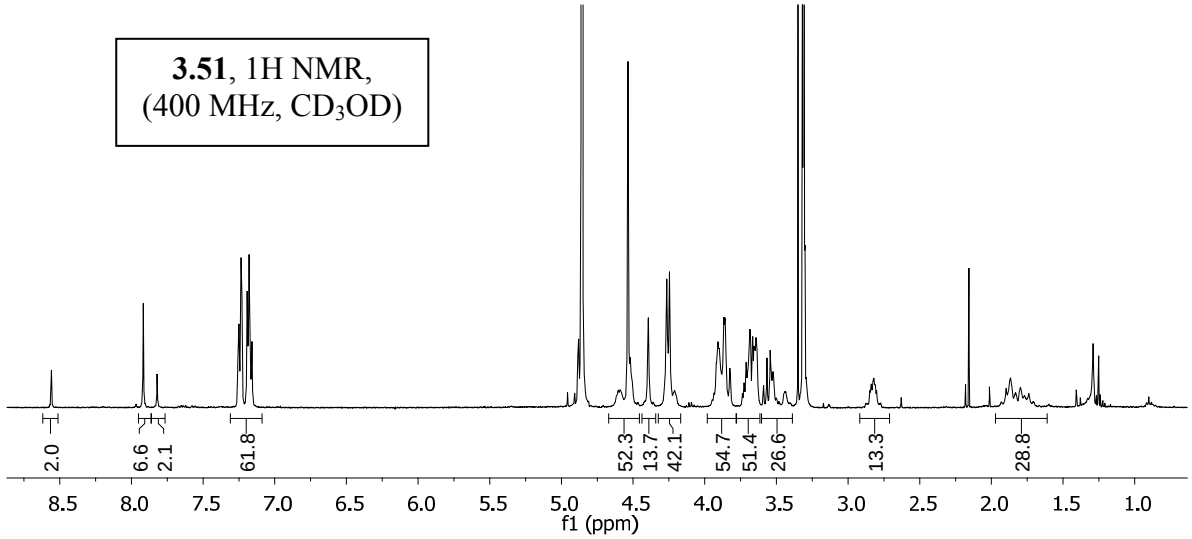
calculated for $[C_{278}H_{370}N_{36}O_{94}]^+$: 5720.1; found = 5719.9

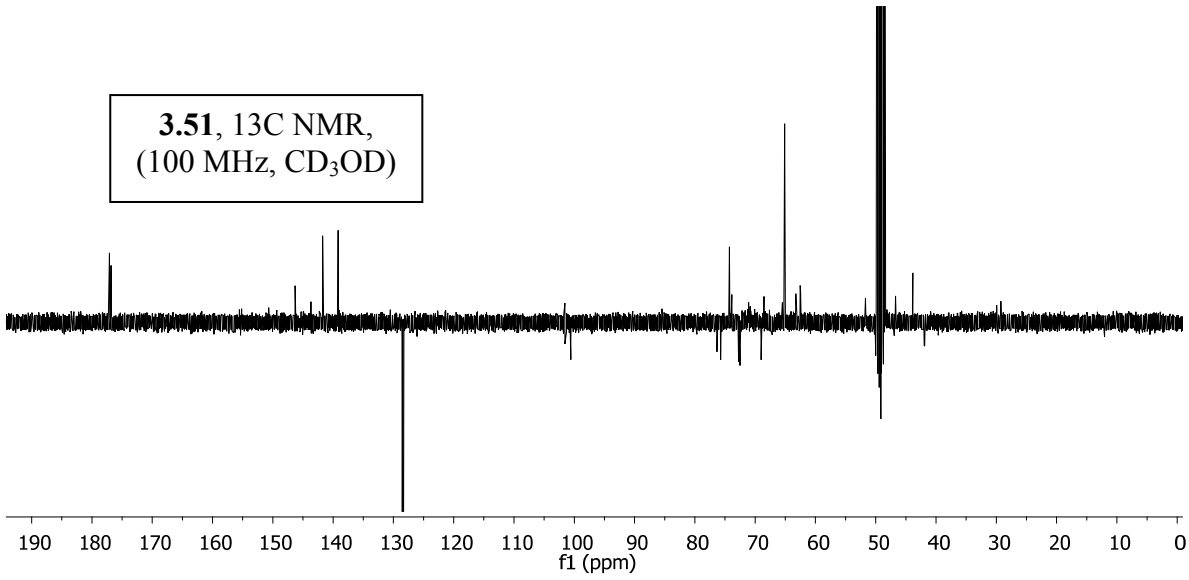
MS (ESI-HRMS): calculated for $[C_{278}H_{370}N_{36}O_{94}]^+$: 5716.52790; found = 5719.52543 (after deconvolution, error: 2.0 ppm)

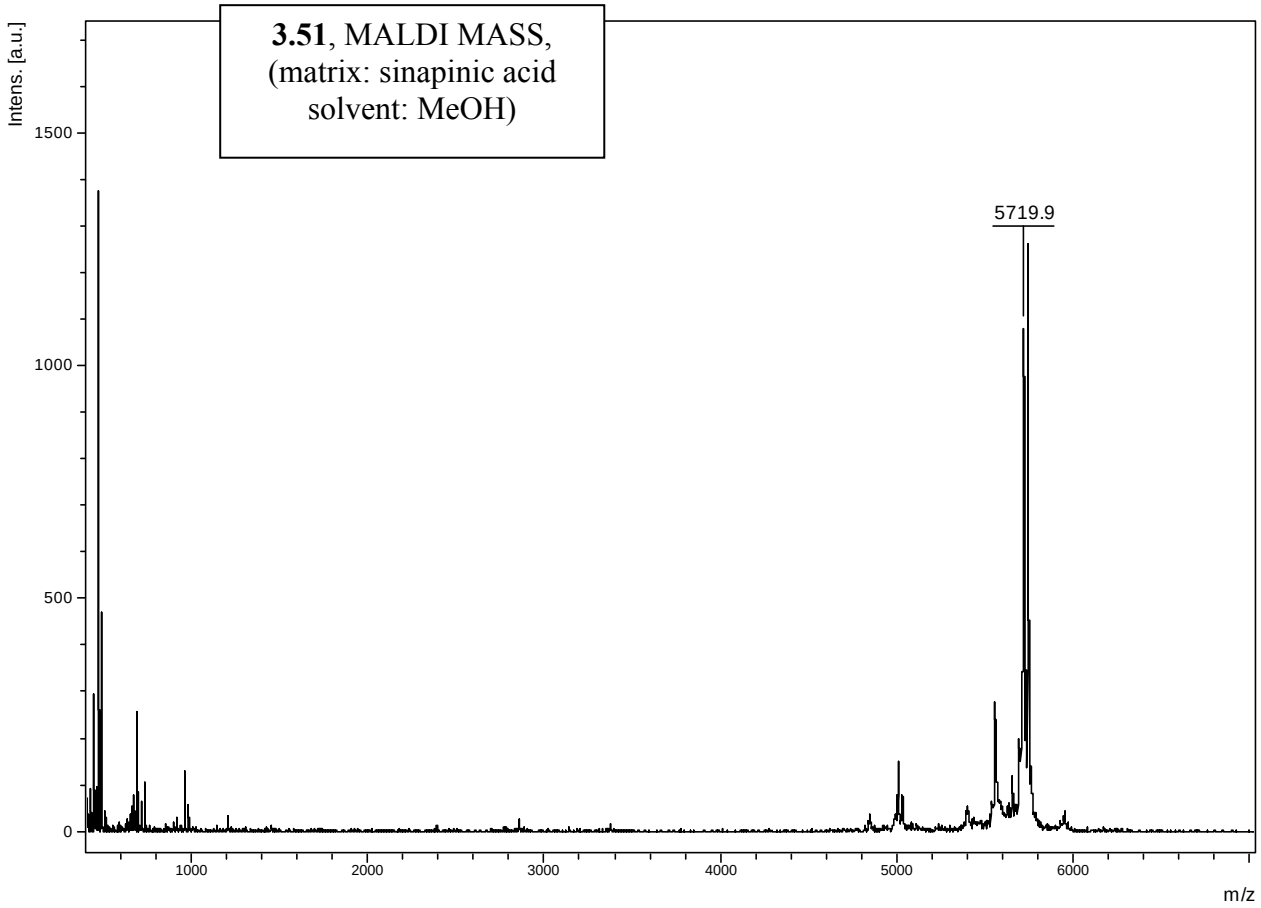
^{1}H NMR (400 MHz, CD_3OD): δ = 8.56 (s, 2H, H_{26}), 7.92 (s, 6H, H_{16}), 7.82 (s, 2H, R_2), 7.28 – 7.13 (m, 52H, H_{12}, H_{13}, R_5, R_{10}), 4.88 (d, 6H, H_1, $J_{1\text{-}2}$ = 1.3 Hz), 4.62 – 4.57 (m, 4H, H_{25}), 4.56 – 4.48 (m, 36H, H_{15}, H_8), 4.39 (s, 12H, H_{18}), 4.32 – 4.17 (m, 36H, H_{10}, G_1, G_5, G_9), 3.95 - 3.79 (m, 46H, H_2, H_{6a}, D_2, H_7, H_{24}, G_2, G_6, G_8), 3.75 - 3.61 (m, 36H, H_{6b}, D_1, H_3, G_3, G_4, G_7, G_8, G_{11}, G_{12}), 3.60 - 3.39 (m, 20H, H_4, H_5, H_{22}, H_{23}), 3.33 – 3.27 (m, 16H, H_{21}, H_{19}), 2.89 – 2.75 (m, 12H, D_4, D_5), 1.96 – 1.66 (m, 24H, D_3, D_6).

^{13}C NMR (100 MHz, CD_3OD): δ = 177.1, 176.8 (C_9); 146.3 (C_{17}); 143.7 (C_{27}); 141.7(C_{14}); 139.2, 139.2 (C_{11}); 128.6, 128.5, 128.3, 128.3 (C_{13}, C_{12}); 126.3 (C_{16}); 126.0 (C_{26}); 119.3 (R_{10}, R_5); 113.5 (R_2); 100.6 (C_1); 76.3 (C_3); 75.7 (C_{D1}); 74.3, 74.3, 73.9 (G_3, G_7, G_{11}); 72.8 (D_2); 72.5, 72.5 (C_2, C_5); 72.3 – 69.0 (C_{22}, C_{23}, C_{21}, C_{19}, G_1, G_5, G_9, G_2, G_6, G_{10}); 69.0 (C_4); 68.5 (C_7); 65.5

(C_{18}); 65.1 (C_{15}); 63.3 (C_6); 62.6, 62.5, 62.5 (G_4, G_8, G_{12}); 51.7 (C_{25}, C_8); 46.7 (C_{20}); 43.9 (C_{10}); 42.0, 41.9 (C_{D4}, C_{D5}); 29.9, 29.2 (C_{D3}, C_{D6}).






3.51, MALDI MASS,
(matrix: sinapinic acid
solvent: MeOH)
Intens. [a.u.]
1500
1000
500
0
5719.9
1000
2000
3000
4000
5000
6000
m/z

3.6 References

1. Gabius, H. J.; Siebert, H. C.; Andre, S.; Jimènez-Barbero, J.; Rudiger, H., Chemical biology of the sugar code. *Chembiochem* **2004,** *5* (6), 740-764.

2. Werz, D. B.; Seeberger, P. H., Chemical Glycomics as Basis for Drug Discovery. In *Chemical Biology*, Wiley-VCH Verlag GmbH: 2008; pp 668-691.

3. Pilobello, K. T.; Mahal, L. K., Deciphering the glycocode: the complexity and analytical challenge of glycomics. *Curr Opin Chem Biol* **2007,** *11* (3), 300-305.

4. Kamerling, J. P., *Comprehensive glycoscience from chemistry to systems biology*. Elsevier: Amsterdam 2007, 2007; Vol. 3, 4.

5. Mammen, M.; Choi, S. K.; Whitesides, G. M., Polyvalent interactions in biological systems: Implications for design and use of multivalent ligands and inhibitors. *Angew Chem Int Edit* **1998,** *37* (20), 2755-2794.

6. Pieters, R. J., Maximising multivalency effects in protein-carbohydrate interactions. *Org Biomol Chem* **2009,** *7* (10), 2013-2025.

7. Kitov, P. I.; Sadowska, J. M.; Mulvey, G.; Armstrong, G. D.; Ling, H.; Pannu, N. S.; Read, R. J.; Bundle, D. R., Shiga-like toxins are neutralized by tailored multivalent carbohydrate ligands. *Nature* **2000,** *403* (6770), 669-672.

8. Rao, J. H.; Whitesides, G. M., Tight binding of a dimeric derivative of vancomycin with dimeric L-Lys-D-Ala-D-Ala. *J Am Chem Soc* **1997,** *119* (43), 10286-10290.

9. Kiessling, L. L.; Gestwicki, J. E.; Strong, L. E., Synthetic multivalent ligands as probes of signal transduction. *Angew Chem Int Edit* **2006,** *45* (15), 2348-2368.

10. Lasala, F.; Arce, E.; Otero, J. R.; Rojo, J.; Delgado, R., Mannosyl glycodendritic structure inhibits DC-SIGN-mediated Ebola virus infection in cis and in trans. *Antimicrob Agents Ch* **2003,** *47* (12), 3970-3972.

11. Bernardi, A.; Cheshev, P., Interfering with the sugar code: Design and synthesis of oligosaccharide mimics. *Chem-Eur J* **2008,** *14* (25), 7434-7441.

12. Luczkowiak, J.; Sattin, S.; Sutkeviciute, I.; Reina, J. J.; Sanchez-Navarro, M.; Thepaut, M.; Martinez-Prats, L.; Daghetti, A.; Fieschi, F.; Delgado, R.; Bernardi, A.; Rojo, J., Pseudosaccharide Functionalized Dendrimers as Potent Inhibitors of DC-SIGN Dependent Ebola Pseudotyped Viral Infection. *Bioconjugate Chem* **2011,** *22* (7), 1354-1365.

13. Wang, S. K.; Liang, P. H.; Astronomo, R. D.; Hsu, T. L.; Hsieh, S. L.; Burton, D. R.; Wong, C. H., Targeting the carbohydrates on HIV-1: Interaction of oligomannose dendrons with human monoclonal antibody 2G12 and DC-SIGN. *P Natl Acad Sci USA* **2008,** *105* (10), 3690-3695.

14. Becer, C. R.; Gibson, M. I.; Geng, J.; Ilyas, R.; Wallis, R.; Mitchell, D. A.; Haddleton, D. M., High-Affinity Glycopolymer Binding to Human DC-SIGN and Disruption of DC-SIGN Interactions with HIV Envelope Glycoprotein. *J Am Chem Soc* **2010,** *132* (43), 15130-15132.

15. Martinez-Avila, O.; Bedoya, L. M.; Marradi, M.; Clavel, C.; Alcami, J.; Penades, S., Multivalent Manno-Glyconanoparticles Inhibit DC-SIGN-Mediated HIV-1 Trans-infection of Human T Cells. *Chembiochem* **2009,** *10* (11), 1806-1809.

16. Reina, J. J.; Sattin, S.; Invernizzi, D.; Mari, S.; Martinez-Prats, L.; Tabarani, G.; Fieschi, F.; Delgado, R.; Nieto, P. M.; Rojo, J.; Bernardi, A., 1,2-mannobioside mimic: Synthesis, DC-SIGN interaction by NMR and docking, and antiviral activity. *Chemmedchem* **2007,** *2* (7), 1030-1036.

17. Maria, S.; Sanchez-Medina, I.; Mereghetti, P.; Belvisi, L.; Jimenez-Barbero, J.; Bernardi, A., Synthesis and conformational analysis of an alpha-D-mannopyranosyl(1 -> 2)-alpha-D-mannopyranosyl-(1 -> 6)-alpha-D-mannopyranose mimic. *Carbohyd Res* **2007,** *342* (12-13), 1859-1868.

18. Rojo, J., Glycosystems Laboratory; Instituto de Investigaciones Químicas, CSIC; Av. Américo Vespucio, 49; Seville 41092; Tel: +34 954489568, FAX: +34 954460565.

19. Xie, J.; Hu, L.; Shi, W.; Deng, X.; Cao, Z.; Shen, Q., Synthesis and nonlinear optical properties of hyperbranched polytriazole containing second-order nonlinear optical chromophore. *Journal of Polymer Science Part B: Polymer Physics* **2008,** *46* (12), 1140-1148.

20. Touaibia, M.; Wellens, A.; Shiao, T. C.; Wang, Q.; Sirois, S.; Bouckaert, J.; Roy, R., Mannosylated G(0) dendrimers with nanomolar affinities to Escherichia coli FimH. *Chemmedchem* **2007,** *2* (8), 1190-1201.

21. Ortega-Munoz, M.; Lopez-Jaramillo, J.; Hernandez-Mateo, F.; Santoyo-Gonzalez, F., Synthesis of glyco-silicas by Cu(I)-catalyzed "click-chemistry" and their applications in affinity chromatography. *Adv Synth Catal* **2006,** *348* (16-17), 2410-2420.

22. Canalle, L. A.; van Berkel, S. S.; de Haan, L. T.; van Hest, J. C. M., Copper-Free Clickable Coatings. *Adv Funct Mater* **2009,** *19* (21), 3464-3470.

23. Tabarani, G.; Thepaut, M.; Stroebel, D.; Ebel, C.; Vives, C.; Vachette, P.; Durand, D.; Fieschi, F., DC-SIGN Neck Domain Is a pH-sensor Controlling Oligomerization SAXS and hydrodynamic studies of extracellular domain. *J Biol Chem* **2009,** *284* (32), 21229-21240.

24. Shelke, S. V.; Cutting, B.; Jiang, X.; Koliwer-Brandl, H.; Strasser, D. S.; Schwardt, O.; Kelm, S.; Ernst, B., A Fragment-Based In Situ Combinatorial Approach To Identify High-Affinity Ligands for Unknown Binding Sites. *Angew Chem Int Ed Engl* **2010,** *49* (33), 5721-5725.

25. (a) Grunder, S.; Muñoz Torres, D.; Marquardt, C.; Błaszczyk, A.; Krupke, R.; Mayor, M., Synthesis and Optical Properties of Molecular Rods Comprising a Central Core-Substituted Naphthalenediimide Chromophore for Carbon Nanotube Junctions. *Eur J Org Chem* **2011,** *2011* (3), 478-496; (b) Andersen, C. S.; Gothelf, K. V., Synthesis of functional molecular rod oligomers. *Org Biomol Chem* **2009,** *7* (1), 58-60 and references 1-2 within it.

26. Vettoretti, G., Unpublished results.

27. Carbohydrate Multivalent System as Tools to Study Pathogen Interactions with DC-SIGN. http://www.carmusys.iiq.csic.es/.

28. Huisgen, R., Cycloadditions — Definition, Classification, and Characterization. *Angew Chem Int Ed Engl* **1968,** *7* (5), 321-328.

29. Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B., A stepwise Huisgen cycloaddition process: Copper(I)-catalyzed regioselective "ligation" of azides and terminal alkynes. *Angew Chem Int Edit* **2002,** *41* (14), 2596-+.

30. Tornoe, C. W.; Christensen, C.; Meldal, M., Peptidotriazoles on solid phase: [1,2,3]-triazoles by regiospecific copper(I)-catalyzed 1,3-dipolar cycloadditions of terminal alkynes to azides. *J Org Chem* **2002,** *67* (9), 3057-3064.

31. Siemsen, P.; Livingston, R. C.; Diederich, F., Acetylenic coupling: A powerful tool in molecular construction. *Angew Chem Int Edit* **2000,** *39* (15), 2633-2657.

32. Saxon, E.; Bertozzi, C. R., Cell surface engineering by a modified Staudinger reaction. *Science* **2000,** *287* (5460), 2007-2010.

33. Kiick, K. L.; Saxon, E.; Tirrell, D. A.; Bertozzi, C. R., Incorporation of azides into recombinant proteins for chemoselective modification by the Staudinger ligation. *P Natl Acad Sci USA* **2002,** *99* (1), 19-24.

34. Brockway, L. O.; Pauling, L., The Electron-Diffraction Investigation of the Structure of Molecules of Methyl Azide and Carbon Suboxide. *Proceedings of the National Academy of Sciences* **1933,** *19* (9), 860-867.

35. Kolb, H. C.; Finn, M. G.; Sharpless, K. B., Click chemistry: Diverse chemical function from a few good reactions. *Angew Chem Int Edit* **2001,** *40* (11), 2004-+.

36. Evans, R. A., The Rise of Azide–Alkyne 1,3-Dipolar 'Click' Cycloaddition and its Application to Polymer Science and Surface Modification. *Aust J Chem* **2007,** *60* (6), 384-395.

37. Touaibia, M.; Roy, R., Glycodendrimers as anti-adhesion drugs against type 1 fimbriated E-coli uropathogenic infections. *Mini-Rev Med Chem* **2007,** *7* (12), 1270-1283.

38. Chan, T. R.; Hilgraf, R.; Sharpless, K. B.; Fokin, V. V., Polytriazoles as copper(I)-stabilizing ligands in catalysis. *Org Lett* **2004,** *6* (17), 2853-2855.

39. LC-MASS analysis conditions: Column: Supelco Ascentis-Express 50x4.6 mm, 2.7 µm, Phase A: Milli-Q water containing 0.05 % (v/v) TFA, Phase B: Acetonitrile (LC-MS grade) containing 0.05 % TFA, Flow: 1 mL/min, partitioned after UV detector (50 % to MS ESI), Temperature: 40°C, UV Detection at 220 and 254 nm with reference at 500 nm (40 nm bandwith), ESI+ detection in the 50-2000 m/z range with alternating MS/MS.

40. Viehe, H. G., *Chemistry of acetylenes*. M. Dekker: New York, 1969; p 366-417.

41. Viehe, H. G., *Chemistry of acetylenes*. M. Dekker: New York, 1969; p 425-588.

42. (a) Ellis, P. J.; Fairlamb, I. J. S.; Hackett, S. F. J.; Wilson, K.; Lee, A. F., Evidence for the Surface-Catalyzed Suzuki-Miyaura Reaction over Palladium Nanoparticles: An Operando XAS Study. *Angew Chem Int Edit* **2010,** *49* (10), 1820-1824; (b) Lambeth, R. H.; Pederson, S. J.; Baranoski, M.; Rawlett, A. M., Methods for Removal of Residual Catalyst from Polymers Prepared by Ring Opening Metathesis Polymerization. *J Polym Sci Pol Chem* **2010,** *48* (24), 5752-5757.

43. (a) Zornik, D.; Meudtner, R. M.; El Malah, T.; Thiele, C. M.; Hecht, S., Designing Structural Motifs for Clickamers: Exploiting the 1,2,3-Triazole Moiety to Generate Conformationally Restricted Molecular Architectures. *Chem-Eur J* **2011,** *17* (5), 1473-1484; (b) Wuttke, E.; Pevny, F.; Hervault, Y. M.; Norel, L.; Drescher, M.; Winter, R. F.; Rigaut, S., Fully Delocalized (Ethynyl)(vinyl)phenylene Bridged Triruthenium Complexes in up to Five Different Oxidation States. *Inorg Chem* **2012,** *51* (3), 1902-1915.

44. (a) Bates, R. W.; Gabel, C. J.; Ji, J. H.; Ramadevi, T., Synthesis of Phenolic Natural-Products Using Palladium-Catalyzed Coupling Reactions. *Tetrahedron* **1995,** *51* (30), 8199-8212; (b) Koradin, C.; Dohle, W.; Rodriguez, A. L.; Schmid, B.; Knochel, P., Synthesis of polyfunctional indoles and related heterocycles mediated by cesium and potassium bases. *Tetrahedron* **2003,** *59* (9), 1571-1587; (c) Anderson, S.; Taylor, P. N.; Verschoor, G. L. B., Benzofuran trimers for organic electroluminescence. *Chem-Eur J* **2004,** *10* (2), 518-527.

45. Kundu, N. G.; Pal, M.; Mahanty, J. S.; Dasgupta, S. K., Palladium-Catalyzed Heteroannulation of Acetylenic-Compounds - a Facile Method for the Synthesis of Benzofurans. *J Chem Soc Chem Comm* **1992,** (1), 41-42.

46. Hiroya, K.; Suzuki, N.; Yasuhara, A.; Egawa, Y.; Kasano, A.; Sakamoto, T., Total syntheses of three natural products, vignafuran, 2-(4-hydroxy-2-methoxyphenyl)-6-methoxybenzofuran-3-carboxylic acid methyl ester, and coumestrol from a common starting material. *J Chem Soc Perk T 1* **2000,** (24), 4339-4346.

47. Ferris, J. P.; Antonucci, F. R., Synthesis of heterocycles by photochemical cyclization of ortho-substituted benzene derivatives. *Journal of the Chemical Society, Chemical Communications* **1972,** (3), 126-127.

48. Glaser, C., Untersuchungen über einige Derivate der Zimmtsäure. *Justus Liebigs Annalen der Chemie* **1870,** *154* (2), 137-171.

49. Glaser, C., Beiträge zur Kenntniss des Acetenylbenzols. *Berichte der deutschen chemischen Gesellschaft* **1869,** *2* (1), 422-424.

50. Fieschi, F., IBS/LPM; 41 rue Jules Horowitz, 38027 Grenoble Cedex 1 - France;Tel : 33 (0)4 38 78 91 77, Fax : 33 (0)4 38 78 54 94.

51. Sattin, S.; Daghetti, A.; Thepaut, M.; Berzi, A.; Sanchez-Navarro, M.; Tabarani, G.; Rojo, J.; Fieschi, F.; Clerici, M.; Bernardi, A., Inhibition of DC-SIGN-Mediated HIV Infection by a Linear Trimannoside Mimic in a Tetravalent Presentation. *Acs Chem Biol* **2010,** *5* (3), 301-312.

52. Fieschi, F. e. a., Unpublished results.

53. Guzzi, C.; Angulo, J.; Nieto, P. M., Unpublished results.

54. Reina, J. J.; Maldonado, O. S.; Tabarani, G.; Fieschi, F.; Rojo, J., Mannose Glycoconjugates Functionalized at Positions 1 and 6. Binding Analysis to DC-SIGN Using Biosensors. *Bioconjugate Chem* **2007,** *18* (3), 963-969.

55. Tabarani, G.; Reina, J. J.; Ebel, C.; Vives, C.; Lortat-Jacob, H.; Rojo, J.; Fieschi, F., Mannose hyperbranched dendritic polymers interact with clustered organization of DC-SIGN and inhibit gp120 binding. *Febs Lett* **2006,** *580* (10), 2402-2408.

56. Huskens, D.; Vermeire, K.; Profy, A. T.; Schols, D., The candidate sulfonated microbicide, PRO 2000, has potential multiple mechanisms of action against HIV-1. *Antivir Res* **2009,** *84* (1), 38-47.

57. Geijtenbeek, T. B. H.; Torensma, R.; van Vliet, S. J.; van Duijnhoven, G. C. F.; Adema, G. J.; van Kooyk, Y.; Figdor, C. G., Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. *Cell* **2000,** *100* (5), 575-585.

58. Hong, P. W. P.; Flummerfelt, K. B.; de Parseval, A.; Gurney, K.; Elder, J. H.; Lee, B., Human immunodeficiency virus envelope (gp120) binding to DC-SIGN and primary dendritic cells is carbohydrate dependent but does not involve 2G12 or cyanovirin binding sites: Implications for structural analyses of gp120-DC-SIGN binding. *J Virol* **2002,** *76* (24), 12855-12865.

59. Lin, G.; Simmons, G.; Pohlmann, S.; Baribaud, F.; Ni, H. P.; Leslie, G. J.; Haggarty, B.; Bates, P.; Weissman, D.; Hoxie, J. A.; Doms, R. W., Differential N-linked glycosylation of human immunodeficiency virus and Ebola virus envelope glycoproteins modulates interactions with DC-SIGN and DC-SIGNR. *J Virol* **2003,** *77* (2), 1337-1346.

60. Sattin, S. Synthesis of inhibitors of DC-SIGN mediated infections - PhD thesis. University Degli Studi di Milano, Milano, 2009.

61. Tassaneetrithep, B.; Burgess, T. H.; Granelli-Piperno, A.; Trumpfherer, C.; Finke, J.; Sun, W.; Eller, M. A.; Pattanapanyasat, K.; Sarasombath, S.; Birx, D. L.; Steinman, R. M.; Schlesinger, S.; Marovich, M. A., DC-SIGN (CD209) mediates dengue virus infection of human dendritic cells. *J Exp Med* **2003,** *197* (7), 823-829.

62. Amara, A., Laboratory 'Biology of Emerging Viruses'; CNRS UMR 7212/INSERM U944; 16 Rue de la Grange Aux Belles; 1st floor; Batiment Hayem; Porte sous Voute; Hopital Saint Louis; 75010 Paris; France.

63. Schaate, A.; Roy, P.; Preuße, T.; Lohmeier, S. J.; Godt, A.; Behrens, P., Porous Interpenetrated Zirconium–Organic Frameworks (PIZOFs): A Chemically Versatile Family of Metal–Organic Frameworks. *Chemistry – A European Journal* **2011,** *17* (34), 9320-9325.

64. Sierra, C. A.; Lahti, P. M., A Simple Multichromophore Design for Energy Transfer in Distyrylbenzenes with Pyrene Pendants. *The Journal of Physical Chemistry A* **2006,** *110* (44), 12081-12088.

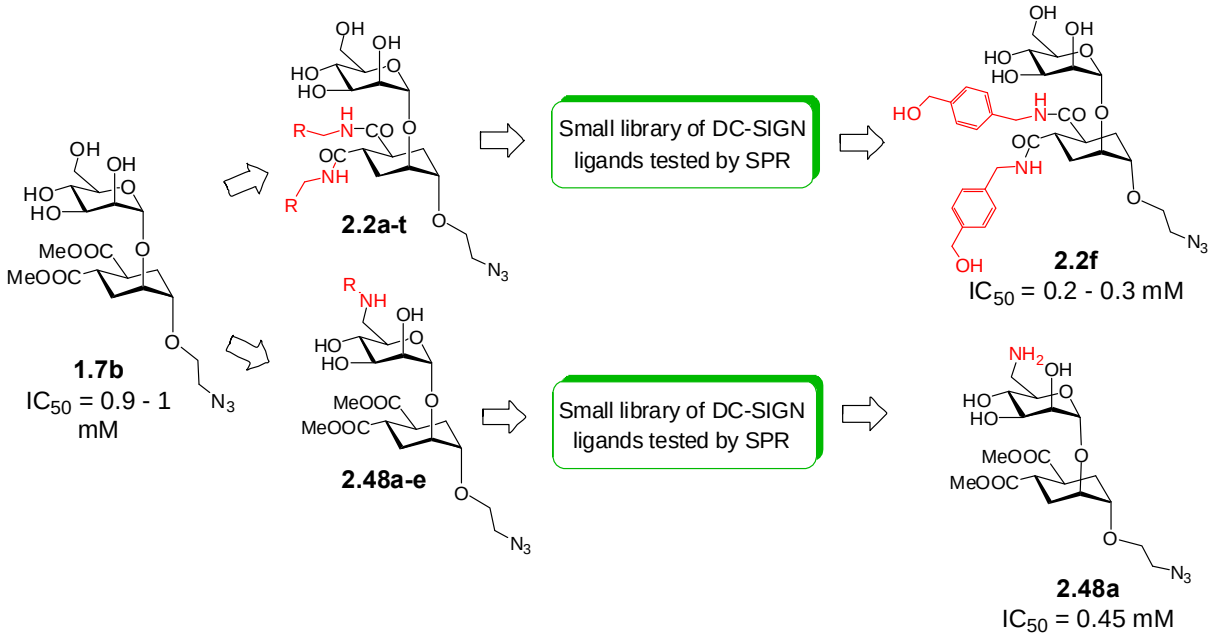
Chapter 4

Conclusions and future plans

In this thesis a development of mannobioside mimetics as potential DC-SIGN inhibitors in mono and multivalent forms is described.

The first part (chapter 2) is focusing on structural optimizations of the previously described pseudodisaccharide **1.7b** (Scheme 4.1). Two major modifications of **1.7b** are discussed. In the first modification the ester groups on the cyclohexyl ring are replaced by two identical amide moieties leading to a focused library of bisamides **2.2a-t**. The best ligands exhibited IC50s between 150-200 µM, which represents an average improvement by a factor of 3 over the parent diester **1.7b**, and compound **2.2f** was selected as a representative of the **2.2** series. Some of the prepared amides were also tested with other lectins besides DC-SIGN to investigate their binding selectivity. It was found that bisamides **2.2f** binds much better DC-SIGN than Langherin in comparison with its counterpart **1.7b**. Preliminary studies also showed that multivalent forms of bisamide **2.2f** have low affinity to the mannose binding lectin (MBL) thus confirming its DC-SIGN specificity.[1] On the other hand, significantly enhanced activity of **2.2f** towards the FimH adhesion protein was found in comparison with the D-mannose monosaccharide.[2] These results showed that the replacement of the methylesters in **1.7b** by aromatic amides can lead to binding specificity of our compounds and further investigations should focus on this issue.

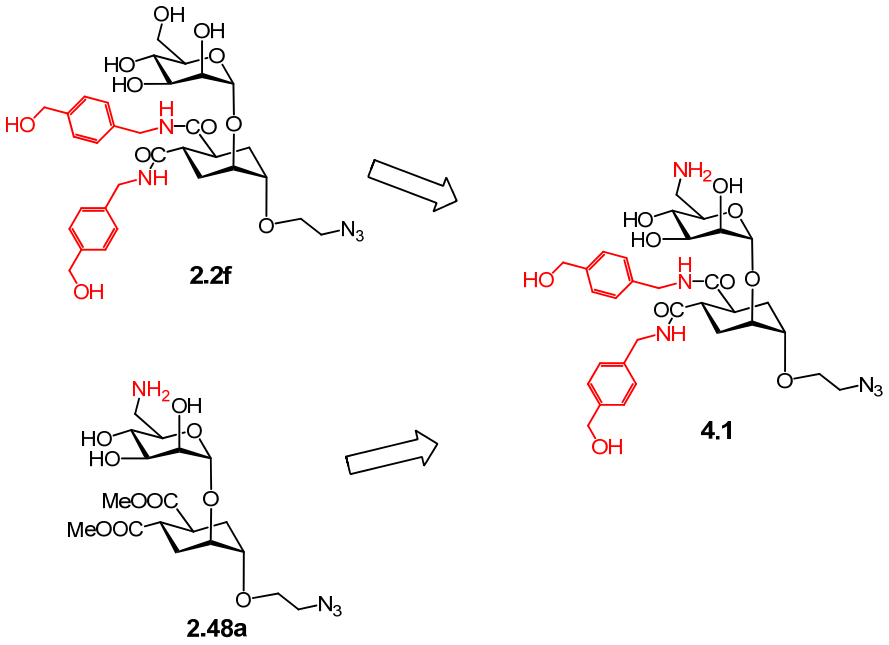
The second modification of **1.7b,** described in the last part of chapter 2, is a replacement of the hydroxyl group in position 6 of the mannose residue. Among the prepared molecules **2.48a-c** and **e**, the primary amine derivative **2.48a** showed an improvement by a factor of 2 in comparison with **1.7b** having the IC50 at 453 µM (Scheme 4.1).



[1] Gobbi, M. et. al., *unpublished results*
[2] Lindhorst, T.; Kolbe, K., *unpublished results*

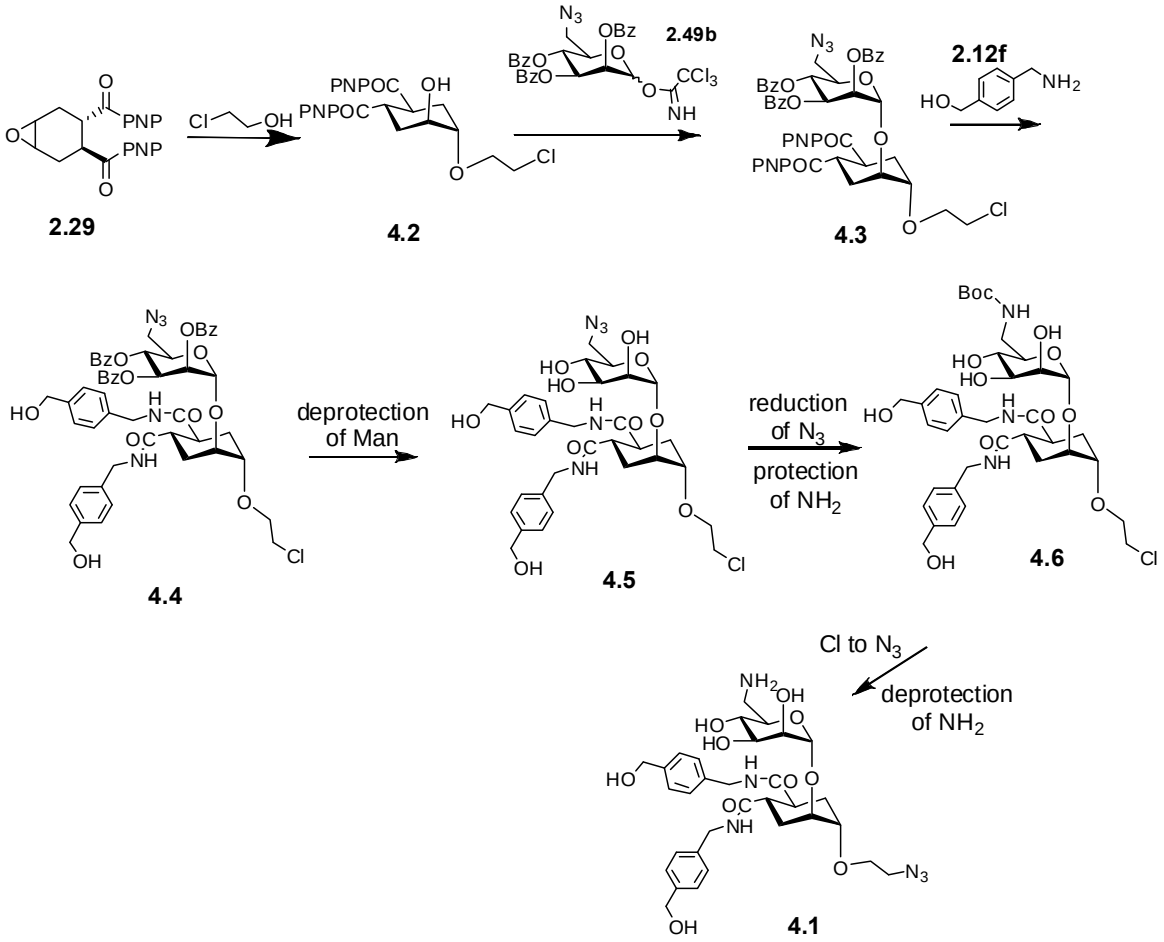
Scheme 4.1 Brief summary of the two modification of **1.7b** described in the second chapter

A merger of **2.2f** and **2.48a** resulting in compound **4.1** could be a target for further investigations.



Scheme 4.2 compound **4.1,** a potential target molecule

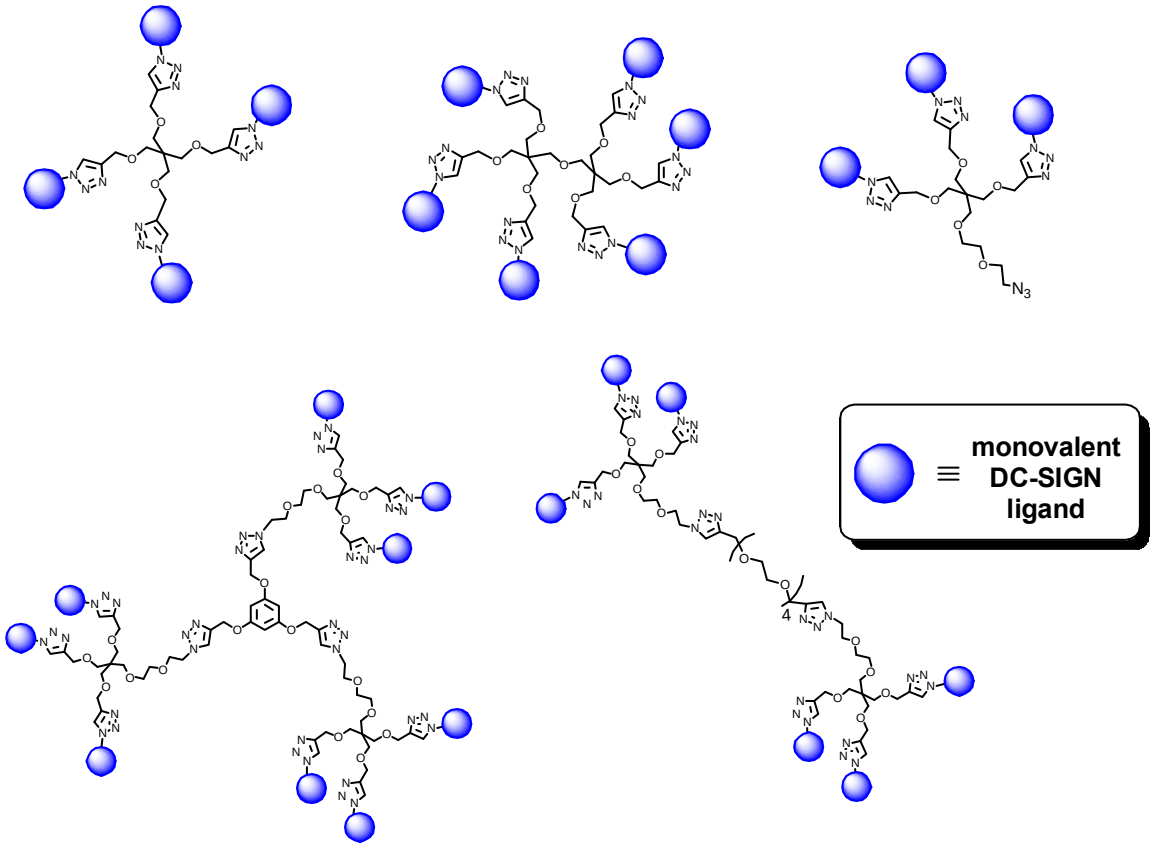
The suggested synthetic strategy for the preparation of **4.1** is a combination of approaches showed in schemes 2.15 and 2.24 (Chapter 2). In the proposed reaction path glycosyl acceptor **4.2** (prepared from epoxide **2.29)** is functionalized with mannose donor **2.49b** bearing an azide in position 6 (Scheme 4.3). The p-nitrophenols in compound **4.3** are substituted with benzylamine derivative **2.12f** followed by deprotection of the benzoyl groups. The azide moiety in **4.5** is reduced and subsequently protected by a Boc group in one pot. In the following steps the chloride in **4.6** is replaced by an azide and the Boc group is cleaved, resulting in the target molecule **4.1** (Scheme 4.3).



Scheme 4.3 The proposed reaction route for the synthesis of **4.1**

In order to predict the activity improvement of the suggested molecule **4.1**, it is important to understand the binding modes of **2.2f** and **2.48a**. If these two molecules interact with the binding site of DC-SIGN in a similar fashion, the fusion of **2.2f** and **2.48a** could lead to a ligand which combines the activity and selectivity improvements of both of these molecules. In order to understand the interaction of our molecules with the binding site of DC-SIGN, structural studies using techniques such as STD–NMR are being carried out by our collaborators within the CARMUSYS network. The upcoming results should answer the questions regarding the binding modes of **2.2f** and **2.48a** and possibly predict the binding behavior of **4.1**.

The second part of my thesis (Chapter 3) focused on the preparation of multivalent constructs decorated with the previously developed monovalent DC-SIGN ligands. The alkyne containing scaffolds **3.1** - **3.5** were functionalized with ligands such as **1.7b**, **2.2f** and **1.9** via the 1,3 dipolar cycloadition (click reaction) leading to a set of multivalent DC-SIGN inhibitors with different valency (Scheme 4.4). The prepared molecules were tested by SPR and HIV infection studies which showed significant multivalency effect.



Scheme 4.4 multivalent constructs described in the third chapter

The most potent ligand **3.23** (hexavalent presentation of **2.2f)** have the IC50 value at the level of 3 μM in the SPR experiment and at 1 μM level in the HIV trans infection studies. This study also led to the first molecule able to inhibit DC-SIGN mediated B-cell infections by Dengue virus. Further, an approach which tries to combine the simultaneous binding of two DC-SIGN binding sites (chelation) with the proximity effects (statistical rebinding) was investigated. Elongated and rigid structures consisting of alkyne-aryl unites were proposed as spacers and prepared. These rod-like molecules were functionalized at both ends with flexible dendrons decorated with 3 copies of **2.2f** (Figure 4.1).

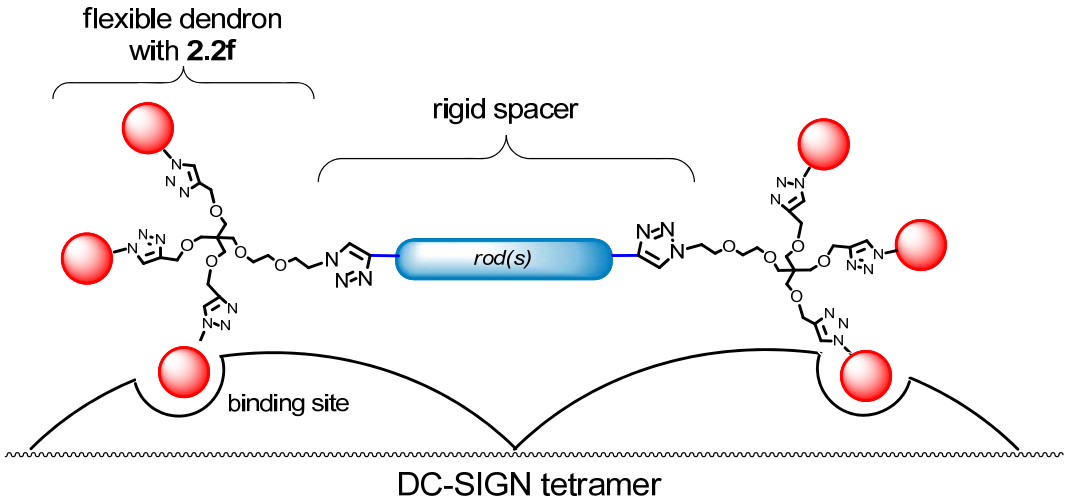
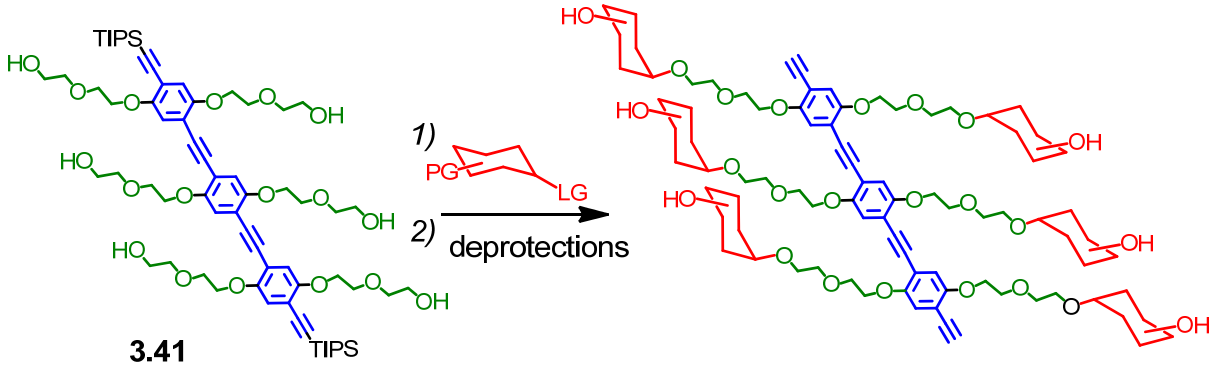


Figure 4.1 Schematic representation of multivalent rod-like structures which are able to inhibit two bindig sites within one DC-SIGN tetramer

The initial experiments showed that these molecules are synthetically achievable in a relatively simple way. The SPR studies indicated higher multivalency effect for the hexavalent rod-like ligand (**3.51)** in comparison with structures having the same valency but lacking the rigid spacer (**3.23,** see Chapter 3, Graph 3.4). The results concerning the development of multivalent rod-like constructs are still preliminary, however the relatively simple synthetic accessibility and the significant multivalency effect are encouraging and further studies should be carried out. During the development of rod-like ligands several issues have been observed. The first of them is related with the low water solubility of the final molecules, whereas the second issue deals with the SPR experimental setup. Some suggestions have been already proposed (section 3.4.2, chapter 3) to overcome these problems. The low water solubility could be solved by the use of more soluble monovalent DC-SIGN ligands such as **1.7b**. Another solution could be a functionalization of the central aromatic rings with highly polar molecules such as monosaccharides, which should improve the water solubility (Scheme 4.5). Moreover, the steric hinderance created by the saccharide residues could prevent aggregations between the aromatic groups of the rod and **2.2f**, and thus keep the molecule elongated and exposed.



Scheme 4.5 Functionalisation of rod **3.41** (Chapter 3) with monosacharides in order to increase its the water solubility

Regarding the activity determination a modification of the SPR experimental setup was proposed in which DC-SIGN is immobilized on the surface of the chip. If the density of the immobilized DC-SIGN is appropriately low, the simultaneous binding showed in figure 4.1 should manifest as a significant increase of the potency of the measured molecules.

The results obtained in this thesis and the suggestions mentioned above, could help to rationalize the design of mono and multivalent DC-SIGN ligands leading to novel and superior inhibitors.

Acknowledgementes

At the end of my thesis I would like to express my gratitude to the people who helped me to achieve the results described in the previous chapters. There are two groups of people to whom I would like thank.

In the first group are those persons who directly participated in the research. First of all I would like to thank to my tutor and supervisor professor Anna Bernardi. I consider myself lucky that I ended up in her group for my PhD thesis for the last three year. She is a bright mind with a lot of experience and knowledge in the field of organic chemistry. Besides that, her communication skills and ability to choose the proper approach to each of her students and co-workers is unique and facilitates the work in the group. Further, I would like to thank to the members of the CARMUSYS network such as Dr. Javier Rojo who is the coordinator of the network and who let me stay in his group for my secondment in Seville. Practically, all my compounds were tested in Grenoble in the research group of professor Franck Fieschi, therefore, his contribution to my thesis is significant. Important structural data, regarding some of the compounds mentioned in this thesis, were obtained in the group of professor Pedro Nieto. The experiments concerning the inhibition of Ebola virus were carried out in the group of Dr. Ali Amara in Paris. Among the PhD students from the network I would like to thank to Ieva Sutkeviciute (Fieschi group) for the SPR results, to Renato Ribeiro (Rojo group) for his help during my secondment, to Cinzia Guzzi (Nieto group) for the structural data and NMR experiments and to Rasika Ramdasi for the Ebola virus infection studies

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parents, through the difficult periods. I have to thank to my brother, Zoli, who is also abroad and for this reason we have very few chances to meet in person these days. Further, there are numerous friends and I would like to mention Gabor, whom I met in Groningen and who is also a ”travelling researcher”, and Jose, a colleague and good friend from Milano.

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Poďakovanie

Na záver mojej prace by som sa chcel poďakovať svojim rodičom, ktorí ma plne podporovali počas môjho doktorandského štúdia. Svojou výchovou ma viedli k hodnotám, ktoré my značne pomohli dosiahnuť moje výsledky. Veľká vďaka patrí aj mojej priateľke a budúcej manželke Edite. Ospravedlňujem sa jej, že som zanechal našu krajinu na tri roky a museli sme udržiavať náš vzťah na diaľku. Napriek tomu stála pri mne a spolu s mojimi rodičmi mi pomáhali riešiť ťažké situácie. Chcel by som sa taktiež poďakovať môjmu bratovi, ktorý je tiež v zahraničí, kvôli čomu máme len málo šancí sa stretnúť osobne.

Vďaka